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**4-Heteroaryl-3-heteroarylidenyl-2-indolinones
And Their Use As Protein Kinase Inhibitors**

CROSS-REFERENCE

5 This application claims priority under 35 U.S.C. 119(e)
to provisional application Serial No. 60/215,654, filed on
June 30, 2000, the disclosure of which is incorporated herein
in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

10 The present invention relates to certain 4-heteroaryl-3-
heteroarylidenyl-2-indolinones compounds and their
physiologically acceptable salts which modulate the activity
15 of protein kinases ("PKs"). The compounds of the present
invention are therefore useful in treating disorders related
to abnormal PK activity. Pharmaceutical composition
containing these compounds and methods of preparing these
compounds are also described.

20 State of the Art

The following is offered as background information only
and is not admitted to be prior art to the present invention.

PKs are enzymes that catalyze the phosphorylation of
25 hydroxy groups on tyrosine, serine and threonine residues of
proteins. The consequences of this seemingly simple activity
are staggering; cell growth, differentiation and
proliferation, i.e., virtually all aspects of cell life in one
way or another depend on PK activity. Furthermore, abnormal
30 PK activity has been related to a host of disorders, ranging
from relatively non life threatening diseases such as
psoriasis to extremely virulent diseases such as glioblastoma
(brain cancer) (see U.S. Patent No. 5,792,783 which is
incorporated herein by reference in its entirety).

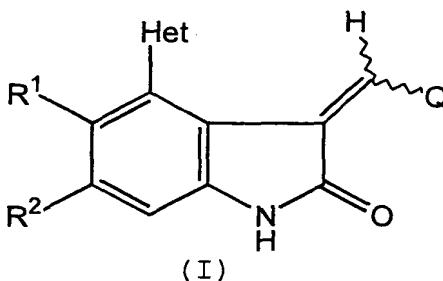
In view of the apparent link between PK-related cellular activities and wide variety of human disorders, a great deal of effort is being expended in an attempt to identify ways to modulate PK activity. Some of this effort has involved biomimetic approaches using large molecules patterned on those involved in the actual cellular processes (e.g., mutant ligands (U.S. Patent No. 4,966,849); soluble receptors and antibodies (Published PCT Appl. WO 94/10202, Kendall and Thomas, Proc. Nat'l Acad. Sci., 90:10705-09 (1994), Kim, et al., Nature, 362:841-844 (1993)); RNA ligands (Jelinek, et al., Biochemistry, 33:10450-56); Takano, et al., Mol. Bio. Cell 4:358A (1993); Kinsella, et al., Exp. Cell Res. 199:56-62 (1992); Wright, et al., J. Cellular Phys., 152:448-57) and tyrosine kinase inhibitors (Published PCT Appls. WO 94/03427; WO 92/21660; WO 91/15495; WO 94/14808; U.S. Patent No. 5,330,992; Mariani, et al., Proc. Am. Assoc. Cancer Res., 35:2268 (1994)).

In addition to the above, attempts have been made to identify small molecules which act as PK inhibitors. For example, bis- monocyclic, bicyclic and heterocyclic aryl compounds (Published PCT Appl. WO 92/20642), vinyleneazaindole derivatives (Published PCT Appl. WO 94/14808) and 1-cyclopropyl-4-pyridylquinolones (U.S. Patent No. 5,330,992) have been described as tyrosine kinase inhibitors. Styryl compounds (U.S. Patent No. 5,217,999), styryl-substituted pyridyl compounds (U.S. Patent No. 5,302,606), quinazoline derivatives (EP App. No.0 566 266 A1), selenaindoles and selenides (Published PCT Appl. WO 94/03427), tricyclic polyhydroxylic compounds (Published PCT Appl. WO 92/21660), benzylphosphonic acid compounds (Published PCT Appl. WO 91/15495) and indolinone compounds (U.S. Patent 5,792,783) have all been described as PTK inhibitors useful in the treatment of cancer. However these compounds have limited utility because of toxicity or poor bioavailability. Accordingly, there is a need for compounds that overcome these

limitations. The compounds of the present invention fulfil this need.

SUMMARY OF THE INVENTION

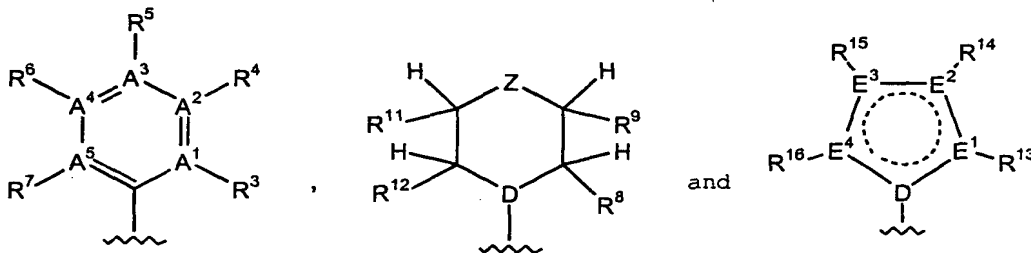
- 5 In one aspect, this invention is directed to a compound of formula (I):



wherein:

- 10 R^1 and R^2 are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, $-CX_3$, hydroxy, alkoxy, nitro, cyano, $-C(O)R^{26}$, $-C(O)OR^{26}$, $R^{26}C(O)O-$, $-C(O)NR^{28}R^{29}$, $R^{26}C(O)NR^{28}-$, $-NR^{28}R^{29}$,
 15 $-S(O)_2R^{26}$, $-S(O)_2OR^{26}$, $-S(O)_2NR^{28}R^{29}$, $R^{26}S(O)_2NR^{28}-$, $X_3CS(O)_2-$ and $X_3CS(O)_2NR^{28}-$ where X is F, Cl, Br, or I;

Het is selected from the group consisting of:



wherein:

- 20 A^1 , A^2 , A^3 , A^4 , and A^5 are selected from the group consisting of carbon and nitrogen with the proviso that at least one and no more than two of A^1 , A^2 , A^3 , A^4 , and A^5 are nitrogen;

R^3 , R^4 , R^5 , R^6 and R^7 are independently selected from the

group consisting of hydrogen, alkyl, halo, hydroxy, alkoxy, X_3C- , nitro, cyano, $-NR^{28}R^{29}$, $-C(O)OR^{26}$ and $-C(O)NR^{28}R^{29}$ where X is as defined above; it being understood that when A^1 , A^2 , A^3 , A^4 or A^5 is nitrogen, R^3 , R^4 , R^5 , R^6 or R^7 , respectively, does
5 not exist;

D is carbon or nitrogen;

R^8 , R^9 , R^{11} and R^{12} are independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo, nitro,
10 cyano and $-NR^{28}R^{29}$;

Z is selected from the group consisting of oxygen, sulfur, and $-NR^{10}$;

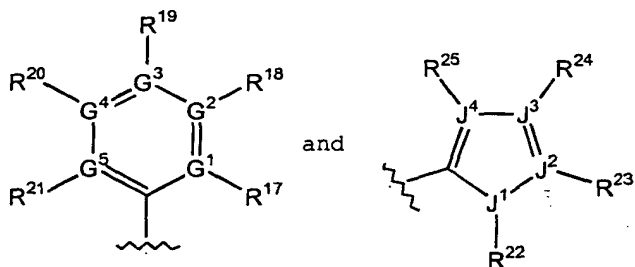
R^{10} is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, $-C(O)R^{26}$, $-C(S)R^{26}$, $-C(O)OR^{26}$, $-$
15 $C(O)NR^{28}R^{29}$, $-C(S)NR^{28}R^{29}$, $-C(NH)NR^{28}R^{29}$ and $-S(O)_2R^{26}$;

E^1 , E^2 , E^3 and E^4 are selected from the group consisting of carbon, nitrogen, oxygen and sulfur with the proviso that when D is carbon then at least one of E^1 , E^2 , E^3 and E^4 is other than carbon and that no more than one of E^1 , E^2 , E^3 or E^4 is oxygen
20 or sulfur;

the dotted circle inside the five-member ring contain D, E^1 , E^2 , E^3 and E^4 ring means that the ring system is aromatic;

R^{13} , R^{14} , R^{15} and R^{16} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl,
25 heteroaryl, heteroalicyclic, hydroxy, alkoxy, mercapto, thioalkoxy, halo, nitro, cyano, $-C(O)R^{26}$, $-C(O)OR^{26}$, $-C(O)NR^{28}R^{29}$ and $-NR^{28}R^{29}$, it being understood that, when one of E^1 , E^2 , E^3 or E^4 is sulfur or oxygen and any of the others is nitrogen, there is no R group bonded to any of those nitrogens, it also being
30 understood that, when two or three of E^1 , E^2 , E^3 or E^4 are nitrogen, there is an R group bonded to one of the nitrogens and that R group is selected from the group consisting of hydrogen and alkyl, there being no R group bonded to any of the other nitrogens;

Q is selected from the group consisting of:



where:

G^1 , G^2 , G^3 , G^4 and G^5 are selected from the group consisting of carbon and nitrogen with the proviso that no more than two of G^1 , G^2 , G^3 , G^4 and G^5 are nitrogen;

R^{17} , R^{18} , R^{19} , R^{20} and R^{21} are independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo,

$-(CH_2)_n C(O)R^{26}$, $-(CH_2)_n C(O)OR^{26}$ and $-(CH_2)_n C(O)NR^{28}R^{29}$, $-(CH_2)_n NR^{28}R^{29}$, $-(CH_2)_n S(O)_2R^{26}$ and $-(CH_2)_n S(O)_2NR^{28}R^{29}$;

J^1 is selected from the group consisting of nitrogen, oxygen and sulfur such that when J^1 is nitrogen, R^{22} is selected from the group consisting of hydrogen, alkyl and $-C(O)R^{26}$; and

when J^1 is oxygen or sulfur, R^{22} does not exist;

J^2 , J^3 and J^4 are selected from the group consisting of carbon and nitrogen;

R^{23} , R^{24} and R^{25} are independently selected from the group consisting of hydrogen, alkyl, aryl optionally substituted with one or more groups independently selected from the group consisting of hydroxy, unsubstituted lower alkoxy and halo, halo,

$-(CH_2)_n C(O)R^{26}$, $-(CH_2)_n C(O)OR^{26}$ and $-(CH_2)_n C(O)NR^{28}R^{29}$, $-(CH_2)_n NR^{28}R^{29}$,

$-(CH_2)_n S(O)_2R^{26}$, $-(CH_2)_n S(O)_2NR^{28}R^{29}$, $-(CH_2)_n OR^{26}$, $-O(CH_2)_n NR^{28}R^{29}$ and $-C(O)NH(CH_2)_n NR^{28}R^{29}$;

n is 0, 1, 2, or 3;

R^{23} and R^{24} or R^{24} and R^{25} may combine to form a group selected from the group consisting of $-CH_2CH_2CH_2CH_2-$, $-CH=CR^{33}-$

CR³⁴=CH- and

-C(O)Y(CH₂)₂- and group wherein Y is selected from the group consisting of oxygen, sulfur and -N(R²⁷)- and R³³ and R³⁴ are

selected from the group consisting of hydrogen, -(CH₂)_nNR²⁸R²⁹

5 and -O(CH₂)_nNR²⁸R²⁹ where, when one of R³³ or R³⁴ is -(CH₂)_nNR²⁸R²⁹

or

-O(CH₂)_nNR²⁸R²⁹, the other is hydrogen;

it being understood that, when J², J³ or J⁴ is nitrogen, R²³, R²⁴

or R²⁵, respectively, does not exist;

10 R²⁶ is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl and heteroaryl;

R²⁷ is selected from the group consisting of hydrogen and alkyl;

R²⁸ and R²⁹ are independently selected from the group

15 consisting of hydrogen, alkyl, aryl, heteroaryl, -(CH₂)_naryl,

-(CH₂)_nheteroaryl and -C(O)R²⁶, or, combined, R²⁸ and R²⁹ may

form a group selected from the group consisting of -(CH₂)₅-,

-(CH₂)₂O(CH₂)₂-, -(CH₂)₂NR³⁰(CH₂)₂- and -(CH₃)₃C(O)- wherein R³⁰ is

selected from the group consisting of hydrogen, alkyl, -

20 C(O)R²⁶, -S(O)₂R²⁶, -S(O)₃R²⁶, -S(O)₂NR³¹R³², -C(O)NHNHNR³¹R³²,

-C(O)NR³¹R³², -C(S)NR³¹R³² and -C(O)OR²⁶ where R³¹ and R³² are

independently selected from the group consisting of hydrogen, unsubstituted lower alkyl and aryl optionally substituted with

one or more groups independently selected from the group

25 consisting of halo and unsubstituted lower alkoxy; or

a pharmaceutically acceptable salt thereof; provided that:

the compound of formula (I) is not:

(Z)-1,3-dihydro-3-[(1H-pyrrol-2-yl)methylene]-4-(2-thiophenyl)-2H-indol-2-one; and

30 Z)-1,3-dihydro-4-(2,4-dimethoxy-6-pyrimidinyl)-3-[(1H-pyrrol-2-yl)methylene]-2H-indol-2-one.

In a second aspect, this invention relates to a method for the modulation of the catalytic activity of a PK by contacting a

PK with a compound of this invention or a pharmaceutically

35 acceptable salt thereof. The modulation of the catalytic activity

of PKs using a compound of this invention may be carried out in vitro or in vivo.

The protein kinase whose catalytic activity is being modulated by a compound of this invention is selected from the group consisting of receptor protein tyrosine kinases, cellular (or non-receptor) tyrosine kinases and serine-threonine kinases.

Preferably, the receptor protein kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of EGF, HER2, HER3, HER4, IR, IGF-1R, IRR, PDGFR α , PDGFR β , CSFIR, C-Kit, C-fms, Flk-1R, Flk4, KDR/Flk-1, Flt-1, FGFR-1R, FGFR-2R, FGFR-3R and FGFR-4R. The cellular tyrosine kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of Src, Frk, Btk, Csk, Abl, ZAP70, Fes/Fps, Fak, Jak, Ack, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk. The serine-threonine protein kinase whose catalytic activity is modulated by a compound of this invention is selected from the group consisting of CDK2 and Raf.

In a third aspect, this invention is directed to a pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier or excipient. Such pharmaceutical composition may contain both carriers and excipients as well as other components generally known to those skilled in the formulation of pharmaceutical compositions.

In a fourth aspect, this invention is directed to a method for treating or preventing a protein kinase related disorder in an organism which method comprises administering to said organism a pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier or excipient.

The above-referenced protein kinase related disorders are those mediated by receptor protein tyrosine kinases, non-receptor or cellular tyrosine kinases, and serine-threonine kinases.

5 Preferably, the protein kinase related disorders are those mediated by EGFR, a PDGFR, IGFR, flk (VEGFR), CDK2, Met kinase, and Src kinase.

10 More preferably, the disorders are cancer selected from the group consisting of squamous cell carcinoma, sarcomas such as Kaposi's sarcoma, astrocytoma, glioblastoma, lung cancer, bladder cancer, colorectal cancer, gastrointestinal cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, breast cancer, small-cell lung cancer, glioma, colorectal cancer, genitourinary cancer and gastrointestinal
15 cancer; diabetic retinopathy, a hyperproliferation disorder, von Hippel-Lindau disease, restenosis, fibrosis, psoriasis, inflammatory disorders such as rheumatoid arthritis, osteoarthritis, immunological disorders such as autoimmune diseases, cardiovascular disorders such as atherosclerosis and
20 angiogenesis related disorders.

In a fifth aspect, this invention is directed to a use of a compound of formula (I) as a reference compound in an assay in order to identify new compounds (test compounds) that modulate protein kinase activity which method comprises
25 contacting cells expressing said protein kinase with a test compound or a compound of formula (I) and then monitoring said cells for an effect.

The above-referenced effect is selected from a change or an absence of change in a cell phenotype, a change or absence
30 of change in the catalytic activity of said protein kinase or a change or absence of change in the interaction of said protein kinase with a natural binding partner.

DETAILED DESCRIPTION OF THE INVENTION

Definitions:

The following terms used in the claims and the specification have the meanings given below. Other terms have their art recognized meaning.

The term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms (whenever a numerical range; e.g. "1-20", is stated herein, it means that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms). More preferably, it is a medium size alkyl radical having 1 to 10 carbon atoms e.g., methyl, ethyl, propyl, 2-propyl, *n*-butyl, *iso*-butyl, *tert*-butyl, pentyl, and the like. Most preferably, it is lower alkyl having 1 to 4 carbon atoms e.g., methyl, ethyl, propyl, 2-propyl, *n*-butyl, *iso*-butyl, or *tert*-butyl, and the like.

The alkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one, two, or three, more preferably one or two groups, independently selected from the group consisting of halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, nitro,

$-C(O)R^{33}$, $-C(S)R^{33}$, $-OC(O)NR^{34}R^{35}$, $R^{33}OC(O)NR^{34}-$, $-OC(S)NR^{34}R^{35}$, $R^{33}OC(S)NR^{34}-$, $-C(O)NR^{34}R^{35}$, $R^{33}C(O)NR^{34}-$, $R^{33}S(O)_2NR^{34}-$, $-S(O)_2NR^{34}R^{35}$, $R^{33}S(O)-$, $R^{33}S(O)_2-$, $-C(O)OR^{33}$, $R^{33}C(O)O-$, $-NR^{34}R^{35}$, aryl optionally substituted with one or more, more preferably one, two, or three groups independently selected from the group consisting of halo, hydroxy and unsubstituted lower alkoxy, aryloxy optionally substituted with one or more, more preferably one, two, or three groups independently selected from the group consisting of halo, hydroxy and unsubstituted lower alkoxy, arylthio optionally substituted with one or more, more preferably one, two, or three groups independently selected from the group consisting of halo, hydroxy and

unsubstituted lower alkoxy, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with one or more, more preferably one or two groups independently selected from the group consisting of halo, hydroxy and unsubstituted lower alkoxy, 5-member heteroaryl having from 1 to 3 heteroatoms in the ring selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms of the group being optionally substituted with one or more, more preferably one or two groups independently selected from the group consisting of halo, hydroxy and unsubstituted lower alkoxy groups and a 5- or 6-member heteroalicyclic group having from 1 to 3 heteroatoms in the ring selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms in the group being optionally substituted with one or more, more preferably one or two groups independently selected from the group consisting of halo, hydroxy and unsubstituted lower alkoxy groups, wherein R^{33} is selected from the group consisting of hydrogen, unsubstituted lower alkyl and aryl optionally substituted with one or more, more preferably one, two, or three groups independently selected from the group consisting of halo and unsubstituted lower alkoxy and R^{34} and R^{35} are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, $-C(O)R^{33}$, aryl optionally substituted with one, two, or three groups independently selected from the group consisting of halo and unsubstituted lower alkoxy and heteroaryl optionally substituted with one, two, or three groups independently selected from the group consisting of halo and unsubstituted lower alkoxy.

A "cycloalkyl" group refers to a 3 to 8 member all-carbon monocyclic ring, an all-carbon 5-member/6-member or 6-member/6-member fused bicyclic ring or a multicyclic fused ring (a "fused" ring system means that each ring in the system shares an adjacent pair of carbon atoms with each other ring in the system) group wherein one or more of the rings may

contain one or more double bonds but none of the rings has a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, adamantane, cycloheptane and, cycloheptatriene. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more independently selected from the group consisting of halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, nitro, $-C(O)R^{33}$, $-C(S)R^{33}$, $-OC(O)NR^{34}R^{35}$, $R^{33}OC(O)NR^{34}-$, $-OC(S)NR^{34}R^{35}$, $R^{33}OC(S)NR^{34}-$, $-C(O)NR^{34}R^{35}$, $R^{33}C(O)NR^{34}-$, $R^{33}S(O)_2NR^{34}-$, $-S(O)_2NR^{34}R^{35}$, $R^{33}S(O)-$, $R^{33}S(O)_2-$, $-C(O)OR^{33}$, $R^{33}C(O)O-$, $-NR^{34}R^{35}$, aryl optionally substituted with one, two or three groups independently selected from the group consisting of halo, hydroxy and unsubstituted lower alkoxy, aryloxy optionally substituted with with one, two or three groups independently selected from the group consisting of halo, hydroxy and unsubstituted lower alkoxy, arylthio optionally substituted with with one, two or three groups independently selected from the group consisting of halo, hydroxy and unsubstituted lower alkoxy, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with with one, two or three groups independently selected from the group consisting of halo, hydroxy and unsubstituted lower alkoxy, 5-member heteroaryl having from 1 to 3 heteroatoms in the ring selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms of the group being optionally substituted with one or two groups independently selected from the group consisting of halo, hydroxy and unsubstituted lower alkoxy groups and a 5- or 6-member heteroalicyclic group having from 1 to 3 heteroatoms in the ring selected from the group consisting of nitrogen, oxygen and sulfur, the carbon atoms in the group being optionally substituted with with one, two or three groups

independently selected from the group consisting of halo, hydroxy and unsubstituted lower alkoxy groups, wherein R^{33} is selected from the group consisting of hydrogen, unsubstituted lower alkyl and aryl optionally substituted with one, two or three groups independently selected from the group consisting of halo and unsubstituted lower alkoxy and R^{34} and R^{35} are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, $-C(O)R^{33}$, aryl optionally substituted with one, two or three groups independently selected from the group consisting of halo and unsubstituted lower alkoxy and heteroaryl optionally substituted with one, two or three groups independently selected from the group consisting of halo and unsubstituted lower alkoxy.

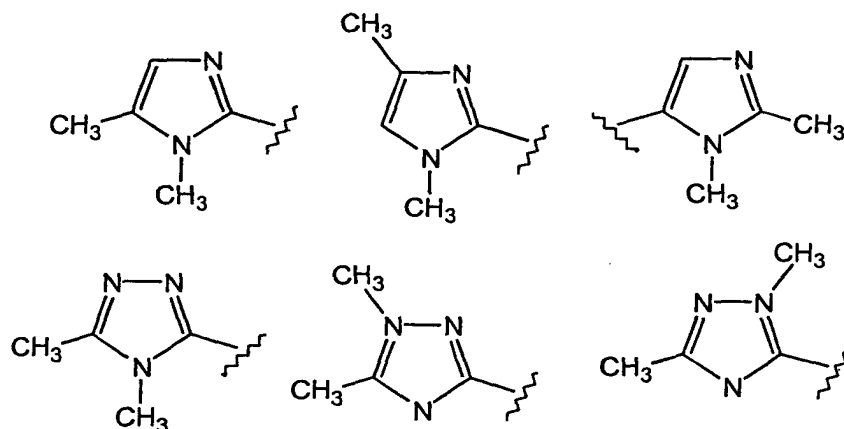
An "alkenyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon double bond e.g., ethenyl, propenyl, butenyl, and the like.

An "alkynyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon triple bond e.g., ethynyl, propynyl, and the like.

An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of carbon atoms) groups of 6 to 12 ring atoms and having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more, more preferably one, two, or three, independently selected from the group consisting of unsubstituted lower alkyl, X_3C- , halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, nitro, $-C(O)R^{33}$, $-C(S)R^{33}$, $-OC(O)NR^{34}R^{35}$, $R^{33}OC(O)NR^{34}-$, $OC(S)NR^{34}R^{35}$, $R^{33}OC(S)NR^{34}-$, $-C(O)NR^{34}R^{35}$, $R^{33}C(O)NR^{34}-$,

$R^{33}S(O)_2NR^{34}-$, $-S(O)_2NR^{34}R^{35}$, $R^{33}S(O)-$, $R^{33}S(O)_2-$, $-C(O)OR^{33}$, $R^{33}C(O)O-$ and $-NR^{34}R^{35}$ with R^{33} , R^{34} and R^{35} as defined above.

As used herein, a "heteroaryl" group refers to a monocyclic or fused aromatic ring (i.e., rings which share an adjacent pair of atoms) containing 5 to 10 ring atoms wherein one, two, three or four ring atoms are independently selected from the group consisting of nitrogen, oxygen and sulfur, the rest being carbon. Examples, without limitation, of heteroaryl groups are pyrrole, furan, thiophene, imidazole, oxazole, isoxazole, thiazole, pyrazole, pyridine, pyrimidine, pyrazine, pyridazine, quinoline, isoquinoline, purine and carbazole. With regard to the five-member heteroaryl groups containing two or more nitrogens and no other hetero atoms in the ring, such as imidazole and triazole, one of the nitrogens in the ring may be bonded to an R group while the others may not. This gives rise to isomeric structures such as those shown below for dimethylimidazoles and dimethyltriazoles:



All such isomers are within the scope of this invention. A heteroaryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more, more preferably one or two groups independently selected from the group consisting of unsubstituted lower alkyl, X_3C- , halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, nitro, $-C(O)R^{33}$, -

$C(S)R^{33}$, $-OC(O)NR^{34}R^{35}$, $R^{33}OC(O)NR^{34}-$, $-OC(S)NR^{34}R^{35}$, $R^{33}OC(S)NR^{34}-$, $-C(O)NR^{34}R^{35}$, $R^{33}C(O)NR^{34}-$, $R^{33}S(O)_2NR^{34}-$, $-S(O)_2NR^{34}R^{35}$, $R^{33}S(O)-$, $R^{33}S(O)_2-$, $-C(O)OR^{33}$, $R^{33}C(O)O-$ and $-NR^{34}R^{35}$ with R^{33} , R^{34} and R^{35} as defined above.

5 A "heteroalicyclic" group refers to a monocyclic or fused ring of 5 to 10 ring atoms wherein one, two, or three ring atoms are independently selected from the group consisting of nitrogen, oxygen and sulfur, the rest being carbon. The rings may also have one or more double bonds. However, the rings do
10 not have a completely conjugated pi-electron system. The heteroalicyclic ring may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more, more preferably one or two groups independently selected from the group consisting of unsubstituted lower
15 alkyl, X_3C- , halo, hydroxy, unsubstituted lower alkoxy, mercapto, (unsubstituted lower alkyl)thio, cyano, nitro, $-C(O)R^{33}$, $-C(S)R^{33}$, $-OC(O)NR^{34}R^{35}$, $R^{33}OC(O)NR^{34}-$, $-OC(S)NR^{34}R^{35}$, $R^{33}OC(S)NR^{34}-$, $-C(O)NR^{34}R^{35}$, $R^{33}C(O)NR^{34}-$, $R^{33}S(O)_2NR^{34}-$, $-S(O)_2NR^{34}R^{35}$, $R^{33}S(O)-$, $R^{33}S(O)_2-$, $-C(O)OR^{33}$, $R^{33}C(O)O-$ and $-NR^{34}R^{35}$ with R^{33} , R^{34} and R^{35} as defined above.
20

 A "hydroxy" group refers to an -OH group.

 An "alkoxy" group refers to both an -O-(unsubstituted alkyl) and an -O-(unsubstituted cycloalkyl) group.

25 An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

 A "mercapto" group refers to an -SH group.

 A "alkylthio" group refers to both an -S(unsubstituted alkyl) and an -S(unsubstituted cycloalkyl) group.

30 A "thioalkoxy" group refers to both an S-alkyl and an -S-cycloalkyl group, as defined herein.

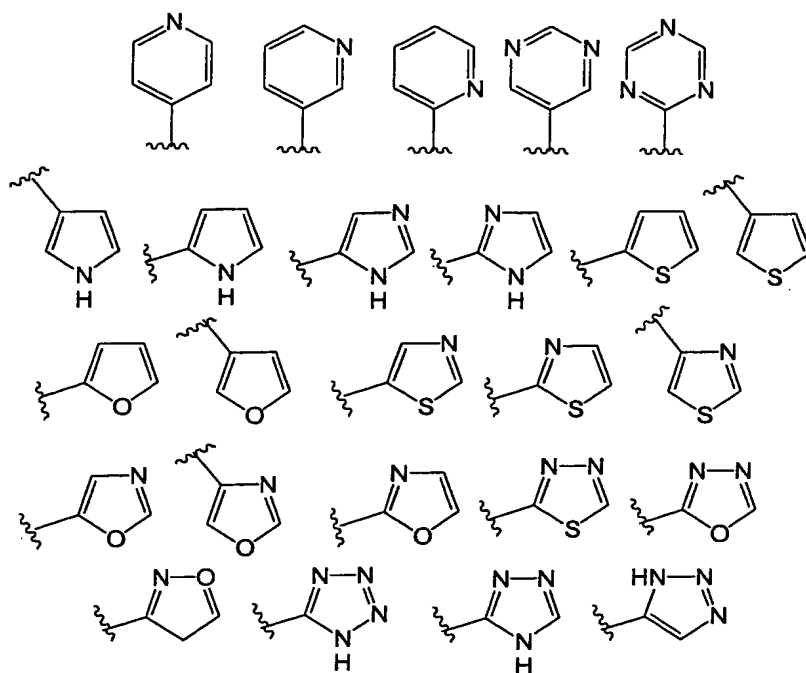
 A "arylthio" group refers to both an -S(aryl) and an -S(heteroaryl group), as defined herein.

 A "halo" group refers to fluorine, chlorine, bromine or iodine.

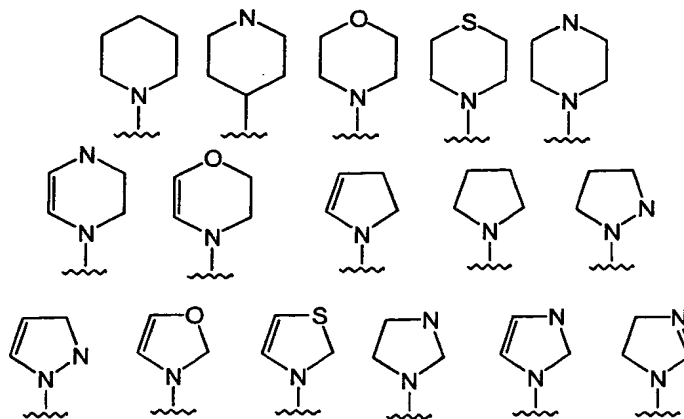
35 A "cyano" group refers to a $-C\equiv N$ group.

A "nitro" group refers to a -NO₂ group.

"Heteroaryl" refers to both heteroaryl groups, defined elsewhere herein and exemplified, without limitation, by the compounds of Group I, below, and heteroalicyclic groups, likewise defined elsewhere herein and, again without limitation, exemplified by the compounds of Group II, below:



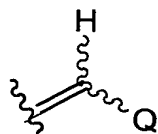
GROUP 1



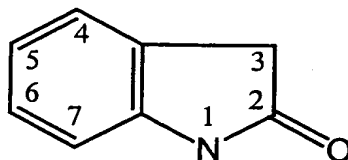
GROUP 2

As used herein "heteroarylidenyl" refers to a group

having the following structure, wherein Q is a heteroaryl group, as defined above.



The terms "2-oxindole," "2-indolinone," and "indolin-2-one" are used interchangeable to refer to a group having the following structure. The 3 and 4 positions, wherein compounds of this invention are substituted with a heteroarylidanyl or a heteroaryl group, respectively, are marked:



As used herein, the term "combined," when referring to two R groups bonded to adjacent carbon atoms, means that the atoms shown as comprising the "combined" structure form a bridge from the carbon to which one of the R groups is bonded to the carbon atom to which the other R group is bonded.

As used herein, "PK" refers to receptor protein tyrosine kinase (RTKs), non-receptor or "cellular" tyrosine kinase (CTKs) and serine-threonine kinases (STKs).

The term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by, practitioners of the chemical, pharmaceutical, biological, biochemical and medical arts.

As used herein, the term "modulation" or "modulating" refers to the alteration of the catalytic activity of RTKs, CTKs and STKs. In particular, modulating refers to the activation or inhibition of the catalytic activity of RTKs, CTKs and STKs,

preferably the activation or inhibition of the catalytic activity of RTKs, CTKs and STKs, depending on the concentration of the compound or salt to which the RTK, CTK or STK is exposed or, more preferably, the inhibition of the catalytic activity of RTKs, CTKs and STKs.

The term "catalytic activity" as used herein refers to the rate of phosphorylation of tyrosine under the influence, direct or indirect, of RTKs and/or CTKs or the phosphorylation of serine and threonine under the influence, direct or indirect, of STKs.

The term "contacting" as used herein refers to bringing a compound of this invention and a target PK together in such a manner that the compound can affect the catalytic activity of the PK, either directly, i.e., by interacting with the kinase itself, or indirectly, i.e., by interacting with another molecule on which the catalytic activity of the kinase is dependent. Such "contacting" can be accomplished "in vitro," i.e., in a test tube, a petri dish or the like. In a test tube, contacting may involve only a compound and a PK of interest or it may involve whole cells. Cells may also be maintained or grown in cell culture dishes and contacted with a compound in that environment.

In this context, the ability of a particular compound to affect a PK related disorder, i.e., the IC_{50} of the compound, defined below, can be determined before use of the compounds in vivo with more complex living organisms is attempted. For cells outside the organism, multiple methods exist, and are well-known to those skilled in the art, to get the PKs in contact with the compounds including, but not limited to, direct cell microinjection and numerous transmembrane carrier techniques.

A "pharmaceutical composition" refers to a mixture of one or more of the compounds described herein, or physiologically/pharmacaceutically acceptable salts or prodrugs thereof, with other chemical components, such as physiologically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

A "prodrug" refers to an agent which is converted into the parent drug in vivo. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a prodrug would be a compound of the present invention which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but then is metabolically hydrolyzed to the carboxylic acid, the active entity, once inside the cell where water solubility is beneficial.

A further example of a prodrug might be a short polypeptide, for example, without limitation, a 2 - 10 amino acid polypeptide, bonded through a terminal amino group to a carboxy group of a compound of this invention wherein the polypeptide is hydrolyzed or metabolized in vivo to release the active molecule.

As used herein, a "physiologically/pharmaceutically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

An "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

"In vitro" refers to procedures performed in an artificial environment such as, e.g., without limitation, in a test tube or culture medium.

As used herein, "in vivo" refers to procedures performed within a living organism such as, without limitation, a mouse, rat or rabbit.

As used herein, "PK related disorder," "PK driven
5 disorder," and "abnormal PK activity" all refer to a condition characterized by inappropriate, i.e., under or, more commonly, over, PK catalytic activity, where the particular PK can be an RTK, a CTK or an STK. Inappropriate catalytic activity can arise as the result of either: (1) PK expression in cells
10 which normally do not express PKs, (2) increased PK expression leading to unwanted cell proliferation, differentiation and/or growth, or, (3) decreased PK expression leading to unwanted reductions in cell proliferation, differentiation and/or growth. Over-activity of a PK refers to either amplification
15 of the gene encoding a particular PK or production of a level of PK activity which can correlate with a cell proliferation, differentiation and/or growth disorder (that is, as the level of the PK increases, the severity of one or more of the symptoms of the cellular disorder increases). Under-activity
20 is, of course, the converse, wherein the severity of one or more symptoms of a cellular disorder increase as the level of the PK activity decreases.

As used herein, the terms "prevent", "preventing" and "prevention" refer to a method for barring an organism from
25 acquiring a PK related disorder in the first place.

As used herein, the terms "treat", "treating" and "treatment" refer to a method of alleviating or abrogating a PK mediated cellular disorder and/or its attendant symptoms. With regard particularly to cancer, these terms simply mean
30 that the life expectancy of an individual affected with a cancer will be increased or that one or more of the symptoms of the disease will be reduced.

The term "organism" refers to any living entity comprised of at least one cell. A living organism can be as simple as,

for example, a single eukariotic cell or as complex as a mammal, such as a cat, dog, human being, etc.

The term "therapeutically effective amount" as used herein refers to that amount of the compound being

5 administered which will relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of cancer, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of the tumor, (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis, (3) inhibiting
10 to some extent (that is, slowing to some extent, preferably stopping) tumor growth, and/or, (4) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the cancer.

15 By "monitoring" is meant observing or detecting the effect of contacting a compound with a cell expressing a particular PK. The observed or detected effect can be a change in cell phenotype, in the catalytic activity of a PK or a change in the interaction of a PK with a natural binding
20 partner. Techniques for observing or detecting such effects are well-known in the art.

"Cell phenotype" refers to the outward appearance of a cell or tissue or the biological function of the cell or tissue. Examples, without limitation, of a cell phenotype are
25 cell size, cell growth, cell proliferation, cell differentiation, cell survival, apoptosis, and nutrient uptake and use. Such phenotypic characteristics are measurable by techniques well-known in the art.

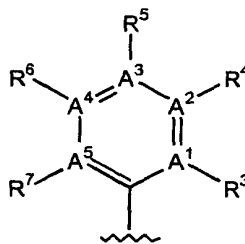
A "natural binding partner" refers to a polypeptide that
30 binds to a particular PK in a cell. Natural binding partners can play a role in propagating a signal in a PK-mediated signal transduction process. A change in the interaction of the natural binding partner with the PK can manifest itself as an increased or decreased concentration of the PK/natural
35 binding partner complex and, as a result, in an observable

change in the ability of the PK to mediate signal transduction.

Preferred Embodiments

5 At present certain compounds of formula (I) are more preferred. Some such preferred embodiments are disclosed below:

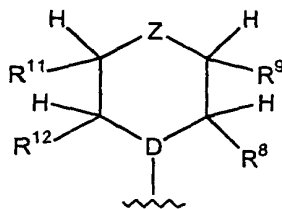
(i) A preferred group of compounds is that wherein Het is:



wherein:

15 A¹ or A² or A³ or A² and A⁴ are nitrogen and the other A's are carbon and the R groups on the A's that are carbon are selected from the group consisting of hydrogen, -NH₂ and -C(O)OR²⁶, R²⁶ being selected from the group consisting of hydrogen and unsubstituted lower alkyl. More preferably Het is 2-, 3-, or 4-pyridyl or 2-, 4-, or 5-pyrimidinyl optionally substituted with an amino or -COOH group. Most preferably 4-pyridyl.

20 (ii) Another presently preferred embodiment of this invention is that wherein Het is:



wherein:

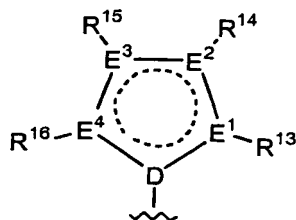
D is nitrogen or carbon, preferably carbon;

R^8 , R^9 , R^{11} and R^{12} are hydrogen; and

Z is $-NR^{10}$ where R^{10} is selected from the group consisting of hydrogen, $-C(O)R^{26}$, $-C(O)OR^{26}$, $-C(O)NR^{28}R^{29}$, $-C(S)NR^{28}R^{29}$, $-C(NH)NR^{28}R^{29}$ and $-S(O)_2R^{26}$ where R^{26} , R^{28} , and R^{29} are as defined in the Summary of the invention. Preferably Het is piperidin-4-yl, piperazin-4-yl, or 4-methylpiperazin-1-yl.

(iii) Another presently preferred aspect of this invention is that wherein Het is:

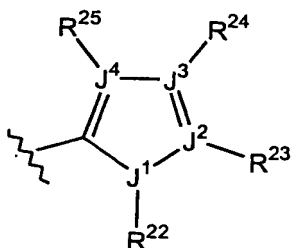
10



wherein:

D is carbon, E¹ is sulfur, E⁴ is nitrogen, E² and E³ are carbon, R¹³ and R¹⁶ do not exist and R¹⁴ and R¹⁵ are hydrogen or E² is nitrogen, E⁴ is sulfur, E¹ and E³ are carbon, R¹³ is hydrogen, R¹⁴ and R¹⁶ do not exist and R¹⁵ is $-NR^{28}R^{29}$ or E² and E³ are nitrogen, E¹ and E⁴ are carbon, R¹³ and R¹⁶ are hydrogen and R¹⁴ and R¹⁵ do not exist. Preferably, Het is thiazol-2-yl.

(iv) Another preferred group of compounds is that wherein Q is:



wherein:

J¹ is nitrogen and J², J³ and J⁴ are carbon.

Within this group a more preferred group of compounds is that wherein R^{22} is hydrogen.

Within the more preferred group, an even more preferred group of compounds is that wherein:

5 R^{23} is selected from the group consisting of hydrogen, unsubstituted lower alkyl, $-C(O)OR^{26}$, and $-C(O)NR^{28}R^{29}$ where R^{26} is hydrogen or unsubstituted lower alkyl and R^{28} and R^{29} are independently selected from the group consisting of hydrogen or unsubstituted lower alkyl or, combined, R^{28} and R^{29} form a
10 group selected from the group consisting of $-(CH_2)_2N(R^{30})(CH_2)_2-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_5-$, R^{30} being selected from the group consisting of hydrogen and unsubstituted lower alkyl. Preferably R^{23} is hydrogen, methyl, ethyl, carboxy,
15 ethoxycarbonyl, pyridin-1-ylcarbonyl, piperazin-1-ylcarbonyl, or 4-methylpiperazin-1-ylcarbonyl; or

R^{23} together with R^{24} combines to form $-(CH_2)_4-$ and $-CH=CH-CR^{34}=CH-$ R^{34} is selected from the group consisting of hydrogen and $-O(CH_2)_2NR^{28}R^{29}$ and R^{28} and R^{29} are independently selected
20 from the group consisting of hydrogen or unsubstituted lower alkyl or, combined, R^{28} and R^{29} form a group selected from the group consisting of $-(CH_2)_2N(R^{30})(CH_2)_2-$, $-(CH_2)_2O(CH_2)_2-$ or $-(CH_2)_5-$, R^{30} being selected from the group consisting of hydrogen and unsubstituted lower alkyl, preferably hydrogen or
25 methyl.

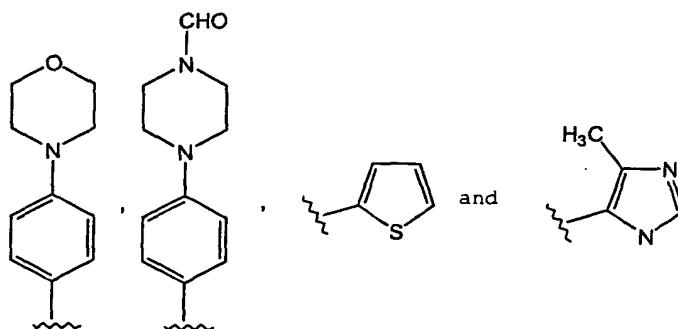
(v) Another preferred group of compounds is that wherein R^{24} and R^{25} are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, aryl optionally substituted with a group selected from the group consisting of
30 halo, unsubstituted lower alkoxy, morpholino and 4-formylpiperidiny, $-(CH_2)_nC(O)NR^{28}R^{29}$, $-(CH_2)_nC(O)OR^{26}$, $-(CH_2)_nNR^{28}R^{29}$, $-(CH_2)_nOR^{26}$, $-C(O)NH(CH_2)_nNR^{28}R^{29}$, $-O(CH_2)_nNR^{28}R^{29}$, $-O(CH_2)_nOR^{26}$ or, combined, a group selected from the group consisting of $-(CH_2)_2OC(O)-$,
35 $-(CH_2)_2N(R^{30})C(O)-$, $-(CH_2)_5-$, $-CH=CH-CH=CH-$ where n is 0 to 3,

R²⁶ is selected from the group consisting of hydrogen and unsubstituted lower alkyl and R²⁸ and R²⁹ are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, lower alkyl substituted with a phenyl or pyridyl group or -NRR where each R is independently hydrogen or unsubstituted lower alkyl; or R²⁸ and R²⁹ combine to form a group selected from the group consisting of -(CH₂)₅-, -(CH₂)₂NR³⁰(CH₂)₂- and -(CH₂)₂O(CH₂)₂- where R³⁰ is selected from the group consisting of hydrogen, unsubstituted lower alkyl and -C(O)R²⁶.

Preferably Q is 3,5-dimethyl-4-(4-methylpiperazin-1-yl-carbonyl)-1H-pyrrol-2-yl, 5-(methyl-3H-imidazol-4-yl)-1H-pyrrol-2-yl, 3-methyl-4-(4-methylpiperidin-1-yl-carbonyl)-1H-pyrrol-2-yl, 3,5-dimethyl-1H-pyrrol-2-yl, 3-(2-carboxyethyl)-4,5,6,7-tetrahydro-1H-indol-2-yl, 3-(2-carboxyethyl)-5-methyl-1H-pyrrol-2-yl, 3-(2-carboxyethyl)-5-ethyl-1H-pyrrol-2-yl, 3-(2-carboxyethyl)-4-ethoxycarbonyl-5-methyl-1H-pyrrol-2-yl, 4-(2-carboxyethyl)-3,5-dimethyl-1H-pyrrol-2-yl, 4-(carboxymethyl)-3,5-dimethyl-1H-pyrrol-2-yl, indol-2-yl, 4,5,6,7-tetrahydroindol-2-yl, 5-(2-morpholin-4-ylethyloxy)indol-2-yl, 3-(carboxy)-5-methyl-1H-pyrrol-2-yl, 5-carboxy-3-methyl-1H-pyrrol-2-yl, 3-(3-morpholin-4-ylpropyl)-4,5,6,7-tetrahydroindol-2-yl, 4-(2-diethylaminoethylaminocarbonyl)-3,5-dimethyl-1H-pyrrol-2-yl, 4-(4-methylpiperazin-1-ylcarbonyl)-3,5-dimethyl-1H-pyrrol-2-yl, 5-(4-methylpiperazin-1-ylcarbonyl)-3-methyl-1H-pyrrol-2-yl, 5-(ethoxycarbonyl)-4,5,6,7-tetrahydro-2H-isoindol-3-yl, 4-(pyridin-4-ylaminocarbonyl)-3-phenyl-5-methyl-1H-pyrrol-2-yl, 5-methylthiophen-2-yl, 3-(2-carboxyethyl)-5-ethoxycarbonyl-4-methyl-1H-pyrrol-2-yl, 3-(2-carboxyethyl)-4-carboxy-1H-pyrrol-2-yl, 3-(4-hydroxyphenyl)-4-ethoxycarbonyl-1H-pyrrol-2-yl, 4-(morpholin-4-ylcarbonyl)-3-methyl-1H-pyrrol-2-yl, 4-(piperidin-1-ylcarbonyl)-3-methyl-1H-pyrrol-2-yl, 3-(2-carboxyethyl)-5-(ethoxycarbonyl)-4-methyl-1H-pyrrol-2-yl, 3-(2-carboxyethyl)-4-(carboxy)-1H-pyrrol-2-yl, 3-(methyl)-4-

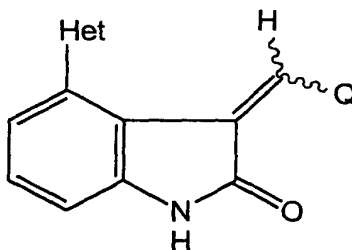
(benzylaminocarbonyl)-1H-pyrrol-2-yl, 3-methyl-4-(pyridin-4-ylmethyloxycarbonyl)-1H-pyrrol-2-yl, 3-methyl-4-[3-(2-oxopyrrolidin-1-yl)propyl-aminocarbonyl]-1H-pyrrol-2-yl, 5-methyl-4-ethoxycarbonyl-3-[3-(4-methylpiperazin-1-yl)propyl]-1H-pyrrol-2-yl, or 3,5-dimethyl-4-(4-methylpiperazin-1-ylaminocarbonyl)-1H-pyrrol-2-yl.

(vi) Yet another preferred group of compounds is that wherein Q is selected from the group consisting of:



10 In the above groups (i-vi), a more preferred group of compounds is that wherein R^1 and R^2 are hydrogen.

(vii) Another preferred group of compounds is represented by the formula (Ia):



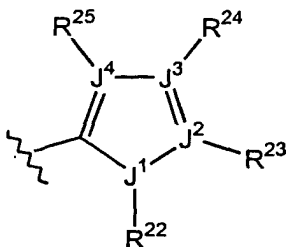
15

wherein:

Het is 2-, 3-, or 4-pyridyl, pyrimidin-5-yl, thiazol-2-yl, or 2-, 3-, or 4-piperidinyl; and

Q is either:

20 (a)



wherein:

J¹ is nitrogen and J², J³ and J⁴ are carbon and other groups are those defined in the Summary of the Invention.

5 Within this group a more preferred group of compounds is that wherein R²² is hydrogen.

Within the more preferred group, an even more preferred group of compounds is that wherein:

10 R²³ is selected from the group consisting of hydrogen, unsubstituted lower alkyl, -C(O)OR²⁶, and -C(O)NR²⁸R²⁹ where R²⁶ is hydrogen or unsubstituted lower alkyl and R²⁸ and R²⁹ are independently selected from the group consisting of hydrogen or unsubstituted lower alkyl or, combined, R²⁸ and R²⁹ form a group selected from the group consisting of -(CH₂)₂N(R³⁰)(CH₂)₂-
15 ,

-(CH₂)₂O(CH₂)₂- or -(CH₂)₅-, R³⁰ being selected from the group consisting of hydrogen and unsubstituted lower alkyl.

20 Preferably R²³ is hydrogen, methyl, ethyl, carboxy, ethoxycarbonyl, pyridin-1-ylcarbonyl, piperazin-1-ylcarbonyl, or 4-methylpiperazin-1-ylcarbonyl; or

25 R²³ together with R²⁴ combines to form -(CH₂)₄- and -CH=CH-CR³⁴=CH- R³⁴ is selected from the group consisting of hydrogen and -O(CH₂)₂NR²⁸R²⁹ and R²⁸ and R²⁹ are independently selected from the group consisting of hydrogen or unsubstituted lower alkyl or, combined, R²⁸ and R²⁹ form a group selected from the group consisting of -(CH₂)₂N(R³⁰)(CH₂)₂-, -(CH₂)₂O(CH₂)₂- or -(CH₂)₅-, R³⁰ being selected from the group consisting of hydrogen and unsubstituted lower alkyl, preferably hydrogen or methyl.

Another even more preferred group of compounds is that wherein R^{24} and R^{25} are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, aryl optionally substituted with a group selected from the group consisting of halo, unsubstituted lower alkoxy, morpholino and 4-formylpiperidinyl, $-(CH_2)_n C(O)NR^{28}R^{29}$, $-(CH_2)_n C(O)OR^{26}$, $-(CH_2)_n NR^{28}R^{29}$, $-(CH_2)_n OR^{26}$, $-C(O)NH(CH_2)_n NR^{28}R^{29}$, $-O(CH_2)_n NR^{28}R^{29}$, $-O(CH_2)_n OR^{26}$ or, combined, a group selected from the group consisting of $-(CH_2)_2 OC(O)-$, $-(CH_2)_2 N(R^{30})C(O)-$, $-(CH_2)_5-$, $-CH=CH-CH=CH-$ where n is 0 to 3, R^{26} is selected from the group consisting of hydrogen and unsubstituted lower alkyl and R^{28} and R^{29} are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, lower alkyl substituted with a phenyl or pyridyl group or $-NRR$ where each R is independently hydrogen or unsubstituted lower alkyl; or R^{28} and R^{29} combine to form a group selected from the group consisting of $-(CH_2)_5-$, $-(CH_2)_2 NR^{30}(CH_2)_2-$ and $-(CH_2)_2 O(CH_2)_2-$ where R^{30} is selected from the group consisting of hydrogen, unsubstituted lower alkyl and $-C(O)R^{26}$.

Preferably Q is 3,5-dimethyl-4-(4-methylpiperazin-1-yl-carbonyl)-1H-pyrrol-2-yl, 5-(methyl-3H-imidazol-4-yl)-1H-pyrrol-2-yl, 3-methyl-4-(4-methylpiperidin-1-yl-carbonyl)-1H-pyrrol-2-yl, 3,5-dimethyl-1H-pyrrol-2-yl, 3-(2-carboxyethyl)-4,5,6,7-tetrahydro-1H-indol-2-yl, 3-(2-carboxyethyl)-5-methyl-1H-pyrrol-2-yl, 3-(2-carboxyethyl)-5-ethyl-1H-pyrrol-2-yl, 3-(2-carboxyethyl)-4-ethoxycarbonyl-5-methyl-1H-pyrrol-2-yl, 4-(2-carboxyethyl)-3,5-dimethyl-1H-pyrrol-2-yl, 4-(carboxymethyl)-3,5-dimethyl-1H-pyrrol-2-yl, indol-2-yl, 4,5,6,7-tetrahydroindol-2-yl, 5-(2-morpholin-4-ylethyloxy)indol-2-yl, 3-(carboxy)-5-methyl-1H-pyrrol-2-yl, 5-carboxy-3-methyl-1H-pyrrol-2-yl, 3-(3-morpholin-4-ylpropyl)-4,5,6,7-tetrahydroindol-2-yl, 4-(2-diethylaminoethylaminocarbonyl)-3,5-dimethyl-1H-pyrrol-2-yl, 4-(4-methylpiperazin-1-ylcarbonyl)-3,5-dimethyl-1H-pyrrol-2-yl, 5-(4-methylpiperazin-1-ylcarbonyl)-3-methyl-1H-pyrrol-2-

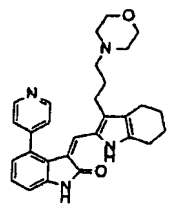
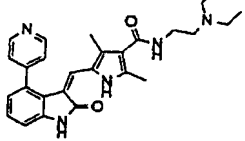
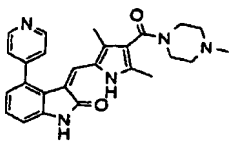
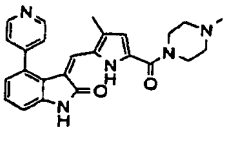
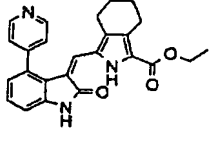
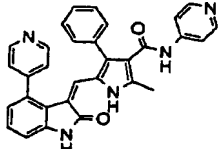
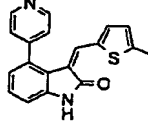
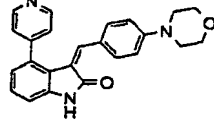
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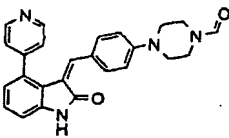
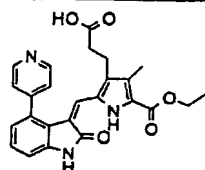
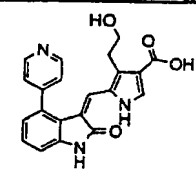
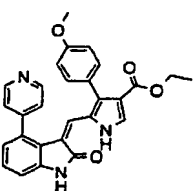
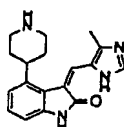
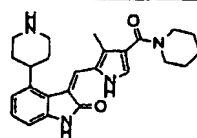
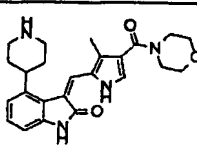
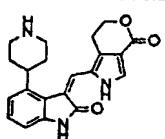
Representative compounds of the invention are disclosed in the Table below:

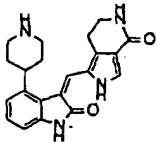
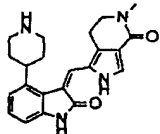
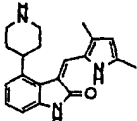
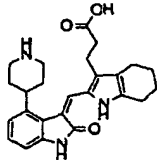
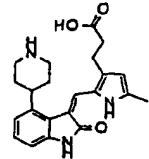
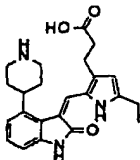
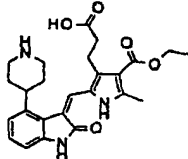
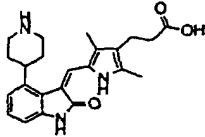
TABLE 1

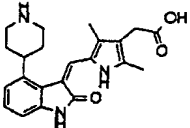
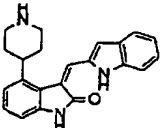
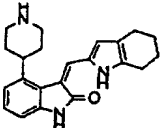
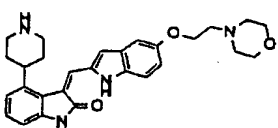
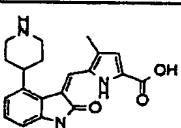
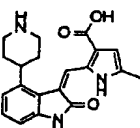
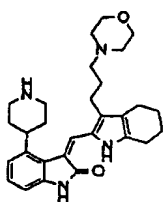
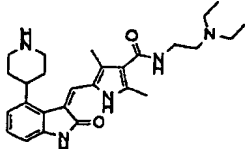
Compound	Structure	Name
1		3-([3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-pyridin-4-yl)-1,3-dihydro-indol-2-one
2		3-(5-Methyl-3H-imidazol-4-ylmethylene)-4-pyridin-4-yl)-1,3-dihydro-indol-2-one
3		1-(2-Oxo-4-pyridin-4-yl)-1,2-dihydro-indol-3-ylidenemethyl)-6,7-dihydro-2H-pyrano[3,4-c]pyrrol-4-one
4		3-(3-Methyl-4-(piperidine-1-carbonyl)-1H-pyrrol-2-ylmethylene)-4-pyridin-4-yl)-1,3-dihydro-indol-2-one
5		3-(3,5-Dimethyl-1H-pyrrol-2-ylmethylene)-4-pyridin-4-yl)-1,3-dihydro-indol-2-one
6		3-(2-(2-Oxo-4-pyridin-4-yl)-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
7		3-(5-Methyl-2-(2-oxo-4-pyridin-4-yl)-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid
8		3-(5-Ethyl-2-(2-oxo-4-pyridin-4-yl)-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid

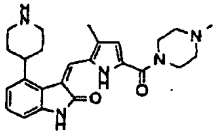
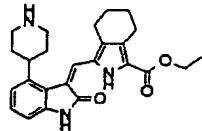
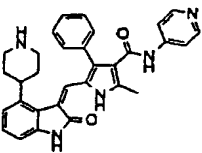
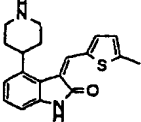
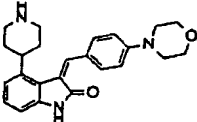
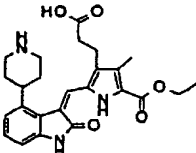
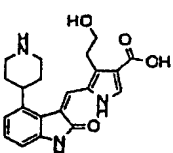
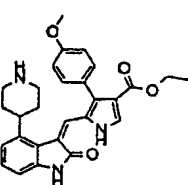
9		4-(2-Carboxy-ethyl)-2-methyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
10		3-[2,4-Dimethyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]propionic acid
11		[2,4-Dimethyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]acetic acid
12		3-(1H-Indol-2-ylmethylene)-4-pyridin-4-yl-1,3-dihydro-indol-2-one
13		4-Pyridin-4-yl-3-(4,5,6,7-tetrahydro-1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one
14		3-[5-(2-Morpholin-4-yl-ethoxy)-1H-indol-2-ylmethylene]-4-pyridin-4-yl-1,3-dihydro-indol-2-one
15		4-Methyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid
16		5-Methyl-2-(2-oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid

17		3-[3-(3-Morpholin-4-yl-propyl)-4,5,6,7-tetrahydro-1H-indol-2-ylmethylene]-4-pyridin-4-yl-1,3-dihydro-indol-2-one
18		2,4-Dimethyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide
19		3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-pyridin-4-yl-1,3-dihydro-indol-2-one
20		3-[3-Methyl-5-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-pyridin-4-yl-1,3-dihydro-indol-2-one
21		3-(2-Oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid ethyl ester
22		2-Methyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-4-phenyl-1H-pyrrole-3-carboxylic acid pyridin-4-ylamide
23		3-(5-Methyl-thiophen-2-ylmethylene)-4-pyridin-4-yl-1,3-dihydro-indol-2-one
24		3-(4-Morpholin-4-yl-benzylidene)-4-pyridin-4-yl-1,3-dihydro-indol-2-one

25		4-[4-(2-Oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-phenyl]-piperazine-1-carbaldehyde
26		4-(2-Carboxy-ethyl)-3-methyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester
27		4-(2-Hydroxy-ethyl)-5-(2-oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid
28		4-(4-Methoxy-phenyl)-5-(2-oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
29		3-(5-Methyl-3H-imidazol-4-ylmethylene)-4-piperidin-4-yl-1,3-dihydro-indol-2-one
30		3-[3-Methyl-4-(piperidine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-piperidin-4-yl-1,3-dihydro-indol-2-one
31		3-[3-Methyl-4-(morpholine-4-carbonyl)-1H-pyrrol-2-ylmethylenel]-4-piperidin-4-yl-1,3-dihydro-indol-2-one
32		1-(2-Oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-6,7-dihydro-2H-pyrano[3,4-c]pyrrol-4-one

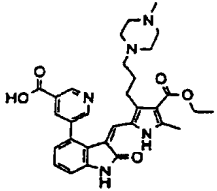
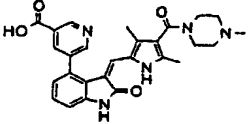
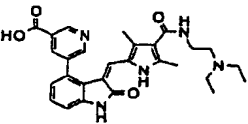
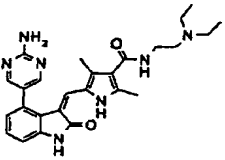
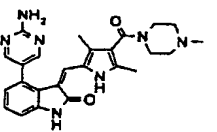
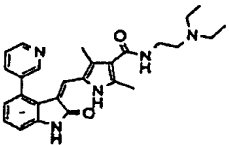
33		1-(2-Oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-2,5,6,7-tetrahydro-pyrrolo[3,4-c]pyridin-4-one
34		5-Methyl-1-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-2,5,6,7-tetrahydro-pyrrolo[3,4-c]pyridin-4-one
35		3-(3,5-Dimethyl-1H-pyrrol-2-ylmethylene)-4-piperidin-4-yl-1,3-dihydro-indol-2-one
36		3-[2-(2-Oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid
37		3-[5-Methyl-2-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid
38		3-[5-Ethyl-2-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid
39		4-(2-Carboxy-ethyl)-2-methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
40		3-[2,4-Dimethyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid

41		[2,4-Dimethyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl] acetic acid
42		3-(1H-Indol-2-ylmethylene)-4-piperidin-4-yl-1,3-dihydro-indol-2-one
43		4-Piperidin-4-yl-3-(4,5,6,7-tetrahydro-1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one
44		3-[5-(2-Morpholin-4-yl-ethoxy)-1H-indol-2-ylmethylene]-4-piperidin-4-yl-1,3-dihydro-indol-2-one
45		4-Methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid
46		5-Methyl-2-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid
47		3-[3-(3-Morpholin-4-yl-propyl)-4,5,6,7-tetrahydro-1H-indol-2-ylmethylene]-4-piperidin-4-yl-1,3-dihydro-indol-2-one
48		2,4-Dimethyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide

49		3-[3-Methyl-5-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-piperidin-4-yl-1,3-dihydro-indol-2-one
50		3-(2-Oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid ethyl ester
51		2-Methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-4-phenyl-1H-pyrrole-3-carboxylic acid pyridin-4-ylamide
52		3-(5-Methyl-thiophen-2-ylmethylene)-4-piperidin-4-yl-1,3-dihydro-indol-2-one
53		3-(4-Morpholin-4-yl-benzylidene)-4-piperidin-4-yl-1,3-dihydro-indol-2-one
54		4-(2-Carboxy-ethyl)-3-methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester
55		4-(2-Hydroxy-ethyl)-5-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid
56		4-(4-Methoxy-phenyl)-5-(2-oxo-4-piperidin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

57		4-Methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid benzylamide
58		4-Methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (pyridin-4-ylmethyl)-amide
59		4-Methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid [3-(2-oxo-pyrrolidin-1-yl)-propyl]-amide
60		2-Methyl-4-[3-(4-methyl-piperazin-1-yl)-propyl]-5-(2-oxo-4-pyridin-2-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
61		3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-pyridin-2-yl-1,3-dihydro-indol-2-one
62		3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-pyrimidin-5-yl-1,3-dihydro-indol-2-one
63		3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-thiazol-2-yl-1,3-dihydro-indol-2-one

64		2-Methyl-4-[3-(4-methyl-piperazin-1-yl)-propyl]-5-(2-oxo-4-pyrimidin-5-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
65		2-Methyl-4-[3-(4-methyl-piperazin-1-yl)-propyl]-5-(2-oxo-4-thiazol-2-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
66		5-[4-(6-Amino-pyridin-3-yl)-2-oxo-1,2-dihydro-indol-3-ylidenemethyl]-2-methyl-4-[3-(4-methyl-piperazin-1-yl)-propyl]-1H-pyrrole-3-carboxylic acid ethyl ester
67		4-(6-Amino-pyridin-3-yl)-3-[3,5-dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-1,3-dihydro-indol-2-one
68		2-Methyl-4-[3-(4-methyl-piperazin-1-yl)-propyl]-5-(2-oxo-4-pyridin-3-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester
69		3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-pyridin-3-yl-1,3-dihydro-indol-2-one

70		5-(3-{4-Ethoxycarbonyl-5-methyl-3-[3-(4-methyl piperazin-1-yl)-propyl]-1H-pyrrol-2-ylmethylene}-2-oxo-2,3-dihydro-1H-indol-4-yl)-nicotinic acid
71		5-{3-[3,5-Dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indol-4-yl}-nicotinic acid
72		5-{3-[4-(2-Diethylamino-ethylcarbamoyl)-3,5-dimethyl-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indol-4-yl}-nicotinic acid
73		5-[4-(2-Amino-pyrimidin-5-yl)-2-oxo-1,2-dihydro-indol-3-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide
74		4-(2-Amino-pyrimidin-5-yl)-3-[3,5-dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-1,3-dihydro-indol-2-one
75		2,4-Dimethyl-5-(2-oxo-4-pyridin-3-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide

1. BRIEF DESCRIPTION OF THE TABLES

TABLE 1 shows the chemical structures of some exemplary compounds of this invention. The compound numbers correspond to the Example numbers in the Examples section. That is, the synthesis of Compound 1 in Table 1 is described in Example 1.

The compounds presented in Table 1 are exemplary only and are not to be construed as limiting the scope of this invention in any manner.

TABLE 2 shows the results of biological testing of some exemplary compounds of this invention. The results are reported in terms of IC_{50} , the micromolar (μM) concentration of the compound being tested which causes a 50% change in the activity of the target PK compared to the activity of the PK in a control to which no test compound has been added. Specifically, the results shown indicate the concentration of a test compound needed to cause a 50% reduction of the activity of the target PK. Bioassays which have been or may be used to evaluate compounds are described in detail below.

Utility

The PKs whose catalytic activity is modulated by the compounds of this invention include protein tyrosine kinases of which there are two types, receptor tyrosine kinases (RTKs) and cellular tyrosine kinases (CTKs), and serine-threonine kinases (STKs). RTK mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), followed by receptor dimerization, transient stimulation of the intrinsic protein tyrosine kinase activity and phosphorylation. Binding sites are thereby created for intracellular signal transduction molecules and lead to the formation of complexes with a spectrum of cytoplasmic signaling molecules that facilitate the appropriate cellular response (e.g., cell division, metabolic effects on the extracellular microenvironment, etc.). See, Schlessinger and Ullrich, 1992, Neuron 9:303-391.

It has been shown that tyrosine phosphorylation sites on growth factor receptors function as high-affinity binding sites for SH2 (src homology) domains of signaling molecules.

Fantl et al., 1992, Cell 69:413-423, Songyang et al., 1994, Mol. Cell. Biol. 14:2777-2785), Songyang et al., 1993, Cell 72:767-778, and Koch et al., 1991, Science 252:668-678.

Several intracellular substrate proteins that associate with RTKs have been identified. They may be divided into two principal groups: (1) substrates that have a catalytic domain, and (2) substrates which lack such domain but which serve as adapters and associate with catalytically active molecules. Songyang et al., 1993, Cell 72:767-778. The specificity of the interactions between receptors and SH2 domains of their substrates is determined by the amino acid residues

immediately surrounding the phosphorylated tyrosine residue. Differences in the binding affinities between SH2 domains and the amino acid sequences surrounding the phosphotyrosine residues on particular receptors are consistent with the observed differences in their substrate phosphorylation profiles. Songyang et al., 1993, Cell 72:767-778. These observations suggest that the function of each RTK is determined not only by its pattern of expression and ligand availability but also by the array of downstream signal transduction pathways that are activated by a particular receptor. Thus, phosphorylation provides an important regulatory step which determines the selectivity of signaling pathways recruited by specific growth factor receptors, as well as differentiation factor receptors.

STKs, being primarily cytosolic, affect the internal biochemistry of the cell, often as a down-line response to a PTK event. STKs have been implicated in the signaling process which initiates DNA synthesis and subsequent mitosis leading to cell proliferation.

A group of STKs that comprise a particularly attractive therapeutic target for cell proliferative disorders are the

cyclin dependent kinases or CDKs. CDKs play a prominent role in control of cellular proliferation. That is, the proliferation of all eukaryotic cells occurs through a continuum of events called the "cell cycle." While in fact a
5 continuum, for purposes of discussion, the cell cycle is conveniently broken down into four phases, G₁, S, G₂ and M. There is another phase, known as G₀, which is not part of the cell cycle per se but rather is a quiescent state in which a cell resides prior to entering the cell cycle at G₁. In G₁,
10 cellular activity is heavily dependent on the stimulating influence of external growth factors. It is during G₁ that the machinery necessary for DNA replication is assembled. Between G₁ and S is a critical point called the "restriction" point. At the restriction point a cell must decide whether it
15 is prepared to continue with the cell cycle. If so, the cell commits to entry into S phase at which time it no longer requires the stimulation of external growth factors. Progress through the cell cycle is entirely intracellular from this point. It is in the S phase that DNA is replicated. At the
20 end of S phase and entry into G₂, a cell has 4N DNA content. In G₂, a cell begins preparation for M phase and cytokinesis. Progression through the cell cycle is regulated by CDKs. As the name suggests, in order to perform their functions, the CDKs require association with cyclin regulatory subunits.
25 Presently, about nine CDKs and about 12 families of cyclins with which the CDKs can interact are known. Two of these, cyclin D/CDK4 and cyclin E/CDK2 are responsible for controlling entry of a cell into G₁ from G₀, passage of the cell through the restriction point and commitment to S phase.
30 Progress through S phase is driven by cyclin E/CDK2 and cyclin A/CDK2, the latter of which promotes completion of S phase and entry into G₂. Finally, progression through G₂, DNA segregation and eventual separation of the parent cell into two daughter cells during M phase and subsequent cytokinesis
35 is controlled by cyclins A and B in conjunction with CDK1.

Throughout the cell cycle there are checkpoints at which a cell monitors both its external and internal environments to assure that continued progress through the cycle is

appropriate. Two important, well-studied check points occur in

5 G1 and G2/mitosis. At the G1 checkpoint, the cell checks to see that it has adequate nutrition, that it is properly

interacting with other cells or their substratum and that its DNA is intact. At the G2 checkpoint, the cell assures that

10 DNA replication is complete and correct and that the mitotic spindle has properly formed. A negative response at any of

these checkpoints results in arrest of the cell cycle which can be temporary, if repairs can be made, or permanent, that is, death of the cell, if repairs cannot be made. These

checkpoints are important because inappropriate cell cycle

15 progress is a hallmark of cell proliferation disorders such as malignant tumor growth. Since CDKs are primarily responsible

for driving cells through the cell cycle, including the checkpoints, their proper functioning is critical to proper cell proliferation. It is for this reason that CDKs have

20 attracted much interest as therapeutic targets. While therapeutic potential exists in all the CDKs, CDK2 has come under particular scrutiny due to the apparently critical role that it play in the cell cycle. For example, it has been demonstrated that CDK2 dominant negative constructs can halt

25 cell cycle progression completely (S. Van den Heuval, et al., *Science*, **1993**, 262:2050-2054). Furthermore, anchorage-

independent growth, a key feature of tumor cells, is mediated by CDK2 complexes (G. Orend, et al., *Oncogene*, **1998**, 16:2575-2583). In another study, a peptide inhibitor of CDK2 function

30 was shown to selectively kill tumor cells over normal cells (Y. N. Chen, et al., *Proc. Natl. Acad. Sci. USA*, **1999**,

96:4221-4223).

While not being bound to any theory, a possible mechanism by which a compound capable of mediating CDK2 function might

35 act can be deduced from the relationship of CDK2 and the tumor

suppression gene p53. If DNA to be replicated has been damaged or if the cell is being stimulated by an oncogene, p53 is activated by the cell and expresses a protein which either suppresses further cell division or simply instructs the cell to kill itself (apoptosis). However, CDK-2 inhibits the activity of p53, thereby keeping it from performing this crucial function and stimulating cell growth. To counter this, p53 protein stimulates the production of another protein, p21, which complexes with CDK2, thereby inactivating it. However, when the p53 gene is damaged (e.g., mutated, a condition found in most tumor types), the p53-p21/CDK2 complex cell/division-inhibition cascade cannot occur and CDK will stimulate the cell, even though damaged, to divide. This can lead to uncontrolled cellular proliferation and cancer. An exogenous CDK2 inhibitor could, in essence, take the place of p53 and prevent the formation of a cancerous tumor. Thus, one aspect of this invention is a compound which inhibits CDK2 function and thereby the formation of malignant tumors.

Thus it can be seen that PK signal transduction results in, among other responses, cell proliferation, differentiation, growth and metabolism. Abnormal cell proliferation may result in a wide array of disorders and diseases, including the development of neoplasia such as carcinoma, sarcoma, glioblastoma and hemangioma, disorders such as leukemia, psoriasis, arteriosclerosis, arthritis and diabetic retinopathy and other disorders related to uncontrolled angiogenesis and/or vasculogenesis.

A precise understanding of the mechanism by which the compounds of this invention inhibit PKs is not required in order to practice the present invention. However, while not hereby being bound to any particular mechanism or theory, it is believed that the compounds interact with the amino acids in the catalytic region of PKs. PKs typically possess a bilobate structure wherein ATP appears to bind in the cleft between the two lobes in a region where the amino acids are

conserved among PKs. Inhibitors of PKs are believed to bind by non-covalent interactions such as hydrogen bonding, van der Waals forces and ionic interactions in the same general region where the aforesaid ATP binds to the PKs. More specifically, it is thought that the 2-indolinone component of the compounds of this invention binds in the region normally occupied by the adenine ring of ATP. Specificity of a particular molecule for a particular PK may then arise as the result of additional interactions between the various substituents on the 2-indolinone core and the amino acid domains specific to particular PKs. Thus, different indolinone substituents may contribute to preferential binding to particular PKs. The ability to select compounds capable of binding to the ATP (or other nucleotide) binding site makes the compounds of this invention useful for targeting any protein with such a site. The compounds disclosed herein may thus have utility as in vitro assays for such proteins as well as exhibiting in vivo therapeutic effects through interaction with such proteins.

In another aspect, the protein kinase, the catalytic activity of which is modulated by contact with a compound of this invention, is a protein tyrosine kinase, more particularly, a receptor protein tyrosine kinase. Among the receptor protein tyrosine kinases whose catalytic activity can be modulated with a compound of this invention, or salt thereof, are, without limitation, EGF, HER2, HER3, HER4, IR, IGF-1R, IRR, PDGFR α , PDGFR β , CSF1R, C-Kit, C-fms, Flk-1R, Flk4, KDR/Flk-1, Flt-1, FGFR-1R, FGFR-2R, FGFR-3R and FGFR-4R.

The protein tyrosine kinase whose catalytic activity is modulated by contact with a compound of this invention, or a salt or a prodrug thereof, can also be a non-receptor or cellular protein tyrosine kinase (CTK). Thus, the catalytic activity of CTKs such as, without limitation, Src, Frk, Btk, Csk, Abl, ZAP70, Fes, Fps, Fak, Jak, Ack, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr and Yrk, may be modulated by contact with a compound or salt of this invention.

Still another group of PKs which may have their catalytic activity modulated by contact with a compound of this invention are the serine-threonine protein kinases such as, without limitation, CDK2 and Raf.

5 This invention is therefore directed to compounds that modulate PK signal transduction by affecting the enzymatic activity of RTKs, CTKs and/or STKs, thereby interfering with the signals transduced by such proteins. More particularly, the present invention is directed to compounds which modulate
10 RTK, CTK and/or STK mediated signal transduction pathways as a therapeutic approach to the treatment of many kinds of solid tumors, including but not limited to carcinomas, sarcomas including Kaposi's sarcoma, erythroblastoma, glioblastoma, meningioma, astrocytoma, melanoma and myoblastoma. Treatment
15 or prevention of non-solid tumor cancers such as leukemia are also contemplated by this invention. Indications may include, but are not limited to brain cancers, bladder cancers, ovarian cancers, gastric cancers, pancreas cancers, colon cancers, blood cancers, lung cancers and bone cancers.

20 Further examples, without limitation, of the types of disorders related to inappropriate PK activity that the compounds described herein may be useful in preventing, treating and studying, are cell proliferative disorders, fibrotic disorders and metabolic disorders.

25 Cell proliferative disorders, which may be prevented, treated or further studied by the present invention include cancer, blood vessel proliferative disorders and mesangial cell proliferative disorders.

Blood vessel proliferative disorders refer to disorders
30 related to abnormal vasculogenesis (blood vessel formation) and angiogenesis (spreading of blood vessels). While vasculogenesis and angiogenesis play important roles in a variety of normal physiological processes such as embryonic development, corpus luteum formation, wound healing and organ
35 regeneration, they also play a pivotal role in cancer

development where they result in the formation of new capillaries needed to keep a tumor alive. Other examples of blood vessel proliferation disorders include arthritis, where new capillary blood vessels invade the joint and destroy cartilage, and ocular diseases, like diabetic retinopathy, where new capillaries in the retina invade the vitreous, bleed and cause blindness.

Two structurally related RTKs have been identified to bind VEGF with high affinity: the fms-like tyrosine 1 (fit-1) receptor (Shibuya et al., 1990, Oncogene, 5:519-524; De Vries et al., 1992, Science, 255:989-991) and the KDR/FLK-1 receptor, also known as VEGF-R2. Vascular endothelial growth factor (VEGF) has been reported to be an endothelial cell specific mitogen with in vitro endothelial cell growth promoting activity. Ferrara & Henzel, 1989, Biochem. Biophys. Res. Comm., 161:851-858; Vaisman et al., 1990, J. Biol. Chem., 265:19461-19566. Information set forth in U.S. application Ser. Nos. 08/193,829, 08/038,596 and 07/975,750, strongly suggest that VEGF is not only responsible for endothelial cell proliferation, but also is the prime regulator of normal and pathological angiogenesis. See generally, Klagsburn & Soker, 1993, Current Biology, 3(10):699-702; Houck, et al., 1992, J. Biol. Chem., 267:26031-26037.

Normal vasculogenesis and angiogenesis play important roles in a variety of physiological processes such as embryonic development, wound healing, organ regeneration and female reproductive processes such as follicle development in the corpus luteum during ovulation and placental growth after pregnancy. Folkman & Shing, 1992, J. Biological Chem., 267(16):10931-34. Uncontrolled vasculogenesis and/or angiogenesis has been associated with diseases such as diabetes as well as with malignant solid tumors that rely on vascularization for growth. Klagsburn & Soker, 1993, Current Biology, 3(10):699-702; Folkham, 1991, J. Natl. Cancer Inst., 82:4-6; Weidner, et al., 1991, New Engl. J. Med., 324:1-5.

The surmised role of VEGF in endothelial cell proliferation and migration during angiogenesis and vasculogenesis indicates an important role for the KDR/FLK-1 receptor in these processes. Diseases such as diabetes mellitus (Folkman, 198, in XIth Congress of Thrombosis and Haemostasis (Verstraeta, et al., eds.), pp. 583-596, Leuven University Press, Leuven) and arthritis, as well as malignant tumor growth may result from uncontrolled angiogenesis. See e.g., Folkman, 1971, N. Engl. J. Med., 285:1182-1186. The receptors to which VEGF specifically binds are an important and powerful therapeutic target for the regulation and modulation of vasculogenesis and/or angiogenesis and a variety of severe diseases which involve abnormal cellular growth caused by such processes. Plowman, et al., 1994, DN&P, 7(6):334-339. More particularly, the KDR/FLK-1 receptor's highly specific role in neovascularization make it a choice target for therapeutic approaches to the treatment of cancer and other diseases which involve the uncontrolled formation of blood vessels.

Thus, one aspect of the present invention relates to compounds capable of regulating and/or modulating tyrosine kinase signal transduction including KDR/FLK-1 receptor signal transduction in order to inhibit or promote angiogenesis and/or vasculogenesis, that is, compounds that inhibit, prevent, or interfere with the signal transduced by KDR/FLK-1 when activated by ligands such as VEGF. Although it is believed that the compounds of the present invention act on a receptor or other component along the tyrosine kinase signal transduction pathway, they may also act directly on the tumor cells that result from uncontrolled angiogenesis.

Although the nomenclature of the human and murine counterparts of the generic "flk-I" receptor differ, they are, in many respects, interchangeable. The murine receptor, Flk-1, and its human counterpart, KDR, share a sequence homology of 93.4% within the intracellular domain. Likewise, murine FLK-I

binds human VEGF with the same affinity as mouse VEGF, and accordingly, is activated by the ligand derived from either species. Millauer et al., 1993, Cell, 72:835-846; Quinn et al., 1993, Proc. Natl. Acad. Sci. USA, 90:7533-7537. FLK-1
5 also associates with and subsequently tyrosine phosphorylates human RTK substrates (e.g., PLC- γ or p85) when co-expressed in 293 cells (human embryonal kidney fibroblasts).

Models which rely upon the FLK-1 receptor therefore are directly applicable to understanding the KDR receptor. For
10 example, use of the murine FLK-1 receptor in methods which identify compounds that regulate the murine signal transduction pathway are directly applicable to the identification of compounds which may be used to regulate the human signal transduction pathway, that is, which regulate
15 activity related to the KDR receptor. Thus, chemical compounds identified as inhibitors of KDR/FLK-1 in vitro, can be confirmed in suitable in vivo models. Both in vivo mouse and rat animal models have been demonstrated to be of excellent value for the examination of the clinical potential of agents
20 acting on the KDR/FLK-1 induced signal transduction pathway.

Thus, in one aspect, this invention is directed to compounds that regulate, modulate and/or inhibit vasculogenesis and/or angiogenesis by affecting the enzymatic activity of the KDR/FLK-1 receptor and interfering with the
25 signal transduced by KDR/FLK-1. In another aspect, the present invention is directed to compounds which regulate, modulate and/or inhibit the KDR/FLK-1 mediated signal transduction pathway as a therapeutic approach to the treatment of many kinds of solid tumors including, but not limited to,
30 glioblastoma, melanoma and Kaposi's sarcoma, and ovarian, lung, mammary, prostate, pancreatic, colon and epidermoid carcinoma. In addition, data suggest the administration of compounds which inhibit the KDR/Flk-1 mediated signal transduction pathway may also be used in the treatment of
35 hemangioma, restenosis and diabetic retinopathy.

A further aspect of this invention relates to the inhibition of vasculogenesis and angiogenesis by other receptor-mediated pathways, including the pathway comprising the flt-1 receptor.

5 Receptor tyrosine kinase mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), followed by receptor dimerization, transient stimulation of the intrinsic protein tyrosine kinase activity and autophosphorylation. Binding sites are thereby created for
10 intracellular signal transduction molecules which leads to the formation of complexes with a spectrum of cytoplasmic signaling molecules that facilitate the appropriate cellular response, e.g., cell division and metabolic effects to the extracellular microenvironment. See, Schlessinger and Ullrich,
15 1992, Neuron, 9:1-20.

The close homology of the intracellular regions of KDR/FLK-1 with that of the PDGF- β receptor (50.3% homology) and/or the related flt-1 receptor indicates the induction of overlapping signal transduction pathways. For example, for the
20 PDGF- β receptor, members of the src family (Twamley et al., 1993, Proc. Natl. Acad. Sci. USA, 90:7696-7700), phosphatidylinositol-3'-kinase (Hu et al., 1992, Mol. Cell. Biol., 12:981-990), phospholipase γ (Kashishian & Cooper, 1993, Mol. Cell. Biol., 4:49-51), ras-GTPase-activating
25 protein, (Kashishian et al., 1992, EMBO J., 11:1373-1382), PTP-ID/syp (Kazlauskas et al., 1993, Proc. Natl. Acad. Sci. USA, 90:6939-6943), Grb2 (Arvidsson et al., 1994, Mol. Cell. Biol., 14:6715-6726), and the adapter molecules Shc and Nck (Nishimura et al., 1993, Mol. Cell. Biol., 13:6889-6896),
30 have been shown to bind to regions involving different autophosphorylation sites. See generally, Claesson-Welsh, 1994, Prog. Growth Factor Res., 5:37-54. Thus, it is likely that signal transduction pathways activated by KDR/FLK-1 include the ras pathway (Rozakis et al., 1992, Nature,

360:689-692), the PI-3'-kinase, the src-mediated and the plcy-mediated pathways. Each of these pathways may play a critical role in the angiogenic and/or vasculogenic effect of KDR/FLK-1 in endothelial cells. Consequently, a still further aspect of this invention relates to the use of the organic compounds described herein to modulate angiogenesis and vasculogenesis as such processes are controlled by these pathways.

Conversely, disorders related to the shrinkage, contraction or closing of blood vessels, such as restenosis, are also implicated and may be treated or prevented by the methods of this invention.

Fibrotic disorders refer to the abnormal formation of extracellular matrices. Examples of fibrotic disorders include hepatic cirrhosis and mesangial cell proliferative disorders. Hepatic cirrhosis is characterized by the increase in extracellular matrix constituents resulting in the formation of a hepatic scar. An increased extracellular matrix resulting in a hepatic scar can also be caused by a viral infection such as hepatitis. Lipocytes appear to play a major role in hepatic cirrhosis. Other fibrotic disorders implicated include atherosclerosis.

Mesangial cell proliferative disorders refer to disorders brought about by abnormal proliferation of mesangial cells. Mesangial proliferative disorders include various human renal diseases such as glomerulonephritis, diabetic nephropathy and malignant nephrosclerosis as well as such disorders as thrombotic microangiopathy syndromes, transplant rejection, and glomerulopathies. The RTK PDGFR has been implicated in the maintenance of mesangial cell proliferation. Floege et al., 1993, Kidney International 43:47S-54S.

Many cancers are cell proliferative disorders and, as noted previously, PKs have been associated with cell proliferative disorders. Thus, it is not surprising that PKs such as, for example, members of the RTK family have been associated with the development of cancer. Some of these

receptors, like EGFR (Tuzi et al., 1991, Br. J. Cancer 63:227-233, Torp et al., 1992, APMIS 100:713-719) HER2/neu (Slamon et al., 1989, Science 244:707-712) and PDGF-R (Kumabe et al., 1992, Oncogene, 7:627-633) are over-expressed in many tumors and/or persistently activated by autocrine loops. In fact, in the most common and severe cancers these receptor over-expressions (Akbasak and Suner-Akbasak et al., 1992, J. Neurol. Sci., 111:119-133, Dickson et al., 1992, Cancer Treatment Res. 61:249-273, Korc et al., 1992, J. Clin. Invest. 90:1352-1360) and autocrine loops (Lee and Donoghue, 1992, J. Cell. Biol., 118:1057-1070, Korc et al., supra, Akbasak and Suner-Akbasak et al., supra) have been demonstrated. For example, EGFR has been associated with squamous cell carcinoma, astrocytoma, glioblastoma, head and neck cancer, lung cancer and bladder cancer. HER2 has been associated with breast, ovarian, gastric, lung, pancreas and bladder cancer. PDGFR has been associated with glioblastoma and melanoma as well as lung, ovarian and prostate cancer. The RTK c-met has also been associated with malignant tumor formation. For example, c-met has been associated with, among other cancers, colorectal, thyroid, pancreatic, gastric and hepatocellular carcinomas and lymphomas. Additionally c-met has been linked to leukemia. Over-expression of the c-met gene has also been detected in patients with Hodgkins disease and Burkitts disease.

IGF-IR, in addition to being implicated in nutritional support and in type-II diabetes, has also been associated with several types of cancers. For example, IGF-I has been implicated as an autocrine growth stimulator for several tumor types, e.g. human breast cancer carcinoma cells (Arteaga et al., 1989, J. Clin. Invest. 84:1418-1423) and small lung tumor cells (Macauley et al., 1990, Cancer Res., 50:2511-2517). In addition, IGF-I, while integrally involved in the normal growth and differentiation of the nervous system, also appears to be an autocrine stimulator of human gliomas. Sandberg-Nordqvist et

al., 1993, Cancer Res. 53:2475-2478. The importance of IGF-IR and its ligands in cell proliferation is further supported by the fact that many cell types in culture (fibroblasts, epithelial cells, smooth muscle cells, T-lymphocytes, myeloid cells, chondrocytes and osteoblasts (the stem cells of the bone marrow)) are stimulated to grow by IGF-I. Goldring and Goldring, 1991, Eukaryotic Gene Expression, 1:301-326. Baserga and Coppola suggest that IGF-IR plays a central role in the mechanism of transformation and, as such, could be a preferred target for therapeutic interventions for a broad spectrum of human malignancies. Baserga, 1995, Cancer Res., 55:249-252, Baserga, 1994, Cell 79:927-930, Coppola et al., 1994, Mol. Cell. Biol., 14:4588-4595.

STKs have been implicated in many types of cancer including, notably, breast cancer (Cance, et al., Int. J. Cancer, 54:571-77 (1993)). The association between abnormal PK activity and disease is not restricted to cancer. For example, RTKs have been associated with diseases such as psoriasis, diabetes mellitus, endometriosis, angiogenesis, atheromatous plaque development, Alzheimer's disease, restenosis, von Hippel-Lindau disease, epidermal hyperproliferation and hemangiomas. For example, EGFR has been indicated in corneal and dermal wound healing. Defects in Insulin-R and IGF-IR are indicated in type-II diabetes mellitus. A more complete correlation between specific RTKs and their therapeutic indications is set forth in Plowman et al., 1994, DN&P 7:334-339.

As noted previously, not only RTKs but CTKs including, but not limited to, src, abl, fps, yes, fyn, lyn, lck, blk, hck, fgr and yrk (reviewed by Bolen et al., 1992, FASEB J., 6:3403-3409) are involved in the proliferative and metabolic signal transduction pathway and thus could be expected, and have been shown, to be involved in many PTK-mediated disorders to which the present invention is directed. For example, mutated src (v-src)

has been shown to be an oncoprotein (pp60^{v-src}) in chicken. Moreover, its cellular homolog, the proto-oncogene pp60^{c-src} transmits oncogenic signals of many receptors. Over-expression of EGFR or HER2/neu in tumors leads to the constitutive
5 activation of pp60^{c-src}, which is characteristic of malignant cells but absent in normal cells. On the other hand, mice deficient in the expression of c-src exhibit an osteopetrotic phenotype, indicating a key participation of c-src in osteoclast function and a possible involvement in related disorders.

10 Similarly, Zap70 has been implicated in T-cell signaling which may relate to autoimmune disorders.

STKs have been associated with inflammation, autoimmune disease, immunoresponses, and hyperproliferation disorders such as restenosis, fibrosis, psoriasis, osteoarthritis and rheumatoid
15 arthritis.

PKs have also been implicated in embryo implantation. Thus, the compounds of this invention may provide an effective method of preventing such embryo implantation and thereby be useful as birth control agents.

20 Finally, both RTKs and CTKs are currently suspected as being involved in hyperimmune disorders.

A method for identifying a chemical compound that modulates the catalytic activity of one or more of the above discussed protein kinases is another aspect of this invention.

25 The method involves contacting cells expressing the desired protein kinase with a compound of this invention (or its salt or prodrug) and monitoring the cells for any effect that the compound has on them. The effect may be any observable, either to the naked eye or through the use of instrumentation, change
30 or absence of change in a cell phenotype. The change or absence of change in the cell phenotype monitored may be, for example, without limitation, a change or absence of change in the catalytic activity of the protein kinase in the cells or a change or absence of change in the interaction of the protein
35 kinase with a natural binding partner.

Examples of the effect of a number of exemplary compounds of this invention on several PKs are shown in Table 2. The compounds and data presented are not to be construed as limiting the scope of this invention in any manner whatsoever.

5

Pharmaceutical Compositions And Use

A compound of the present invention, a prodrug thereof or a physiologically acceptable salt of either the compound or its prodrug, can be administered as such to a human patient or can be administered in pharmaceutical compositions in which the foregoing materials are mixed with suitable carriers or excipient(s). Techniques for formulation and administration of drugs may be found in *Remington's Pharmacological Sciences*, Mack Publishing Co., Easton, PA, latest edition.

Routes of Administration:

As used herein, "administer" or "administration" refers to the delivery of a compound, salt or prodrug of the present invention or of a pharmaceutical composition containing a compound, salt or prodrug of this invention to an organism for the purpose of prevention or treatment of a PK-related disorder.

Suitable routes of administration may include, without limitation, oral, rectal, transmucosal or intestinal administration or intramuscular, subcutaneous, intramedullary, intrathecal, direct intraventricular, intravenous, intravitreal, intraperitoneal, intranasal, or intraocular injections. The preferred routes of administration are oral and parenteral.

Alternatively, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a solid tumor, often in a depot or sustained release formulation.

Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with

tumor-specific antibody. The liposomes will be targeted to and taken up selectively by the tumor.

Composition/Formulation:

Pharmaceutical compositions of the present invention may
5 be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with
10 the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent
15 upon the route of administration chosen.

For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal
20 administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated by combining the active compounds with pharmaceutically
25 acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, lozenges, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient. Pharmaceutical preparations for oral use can be
30 made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding other suitable auxiliaries if desired, to obtain tablets or dragee cores. Useful excipients are, in particular, fillers such as sugars, including lactose,
35 sucrose, mannitol, or sorbitol, cellulose preparations such

as, for example, maize starch, wheat starch, rice starch and potato starch and other materials such as gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinyl-pyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid. A salt such as sodium alginate may also be used.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with a filler such as lactose, a binder such as starch, and/or a lubricant such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. Stabilizers may be added in these formulations, also.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray using a pressurized pack or a nebulizer and a suitable propellant, e.g., without limitation, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetra-fluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be controlled by providing a valve to deliver a metered amount. Capsules and

cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may also be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating materials such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of a water soluble form, such as, without limitation, a salt, of the active compound. Additionally, suspensions of the active compounds may be prepared in a lipophilic vehicle. Suitable lipophilic vehicles include fatty oils such as sesame oil, synthetic fatty acid esters such as ethyl oleate and triglycerides, or materials such as liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers and/or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as depot preparations. Such long acting formulations may be administered by implantation

(for example, subcutaneously or intramuscularly) or by intramuscular injection. A compound of this invention may be formulated for this route of administration with suitable polymeric or hydrophobic materials (for instance, in an emulsion with a pharmacologically acceptable oil), with ion exchange resins, or as a sparingly soluble derivative such as, without limitation, a sparingly soluble salt.

A non-limiting example of a pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer and an aqueous phase such as the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:D5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of such a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of Polysorbate 80, the fraction size of polyethylene glycol may be varied, other biocompatible polymers may replace polyethylene glycol, e.g., polyvinyl pyrrolidone, and other sugars or polysaccharides may substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. In addition, certain organic solvents such as dimethylsulfoxide also may be employed, although often at the cost of greater toxicity.

Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of

solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions herein also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Many of the PK modulating compounds of the invention may be provided as physiologically acceptable salts wherein the claimed compound may form the negatively or the positively charged species. Examples of salts in which the compound forms the positively charged moiety include, without limitation, quaternary ammonium (defined elsewhere herein), salts such as the hydrochloride, sulfate, carbonate, lactate, tartrate, maleate, succinate wherein the nitrogen atom of the quaternary ammonium group is a nitrogen of the selected compound of this invention which has reacted with the appropriate acid. Salts in which a compound of this invention forms the negatively charged species include, without limitation, the sodium, potassium, calcium and magnesium salts formed by the reaction of a carboxylic acid group in the compound with an appropriate base (e.g. sodium hydroxide (NaOH), potassium hydroxide (KOH), Calcium hydroxide (Ca(OH)₂), etc.).

Dosage:

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an amount sufficient to achieve

the intended purpose, e.g., the modulation of PK activity or the treatment or prevention of a PK-related disorder.

More specifically, a therapeutically effective amount means an amount of compound effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from cell culture assays. Then, the dosage can be formulated for use in animal models so as to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal inhibition of the PK activity). Such information can then be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the IC_{50} and the LD_{50} (both of which are discussed elsewhere herein) for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active species which are sufficient to maintain the kinase modulating effects. These

plasma levels are referred to as minimal effective concentrations (MECs). The MEC will vary for each compound but can be estimated from in vitro data, e.g., the concentration necessary to achieve 50-90% inhibition of a kinase may be ascertained using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen that maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.

In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration and other procedures known in the art may be employed to determine the correct dosage amount and interval.

The amount of a composition administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Packaging:

The compositions may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

The pack or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or of human or veterinary administration. Such notice, for example, may be of the labeling approved by the U.S. Food and Drug

Administration for prescription drugs or of an approved product insert. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and
5 labeled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of a tumor, inhibition of angiogenesis, treatment of fibrosis, diabetes, and the like.

It is also an aspect of this invention that a compound
10 described herein, or its salt or prodrug, might be combined with other chemotherapeutic agents or cyclooxygenase-2 (COX-2) inhibitors for the treatment of the diseases and disorders discussed above. For instance, a compound, salt or prodrug of this invention might be combined with alkylating agents such as
15 fluorouracil (5-FU) alone or in further combination with leukovorin; or other alkylating agents such as, without limitation, other pyrimidine analogs such as UFT, capecitabine, gemcitabine and cytarabine, the alkyl sulfonates, e.g., busulfan (used in the treatment of chronic granulocytic
20 leukemia), improsulfan and piposulfan; aziridines, e.g., benzodepa, carboquone, meturedopa and uredepa; ethyleneimines and methylmelamines, e.g., altretamine, triethylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate and trimethylolmelamine; and the nitrogen mustards, e.g.,
25 chlorambucil (used in the treatment of chronic lymphocytic leukemia, primary macroglobulinemia and non-Hodgkin's lymphoma), cyclophosphamide (used in the treatment of Hodgkin's disease, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, Wilm's tumor and
30 rhabdomyosarcoma), estramustine, ifosfamide, novembrichin, prednimustine and uracil mustard (used in the treatment of primary thrombocytosis, non-Hodgkin's lymphoma, Hodgkin's disease and ovarian cancer); and triazines, e.g., dacarbazine (used in the treatment of soft tissue sarcoma).

Likewise a compound, salt or prodrug of this invention might be expected to have a beneficial effect in combination with other antimetabolite chemotherapeutic agents such as, without limitation, folic acid analogs, e.g. methotrexate

5 (used in the treatment of acute lymphocytic leukemia, choriocarcinoma, mycosis fungoides breast cancer, head and neck cancer and osteogenic sarcoma) and pteropterin; and the purine analogs such as mercaptopurine and thioguanine which find use in the treatment of acute granulocytic, acute
10 lymphocytic and chronic granulocytic leukemias.

A compound, salt or prodrug of this invention might also be expected to prove efficacious in combination with natural product based chemotherapeutic agents such as, without limitation, the vinca alkaloids, e.g., vinblastin (used in the
15 treatment of breast and testicular cancer), vincristine and vindesine; the epipodophylotoxins, e.g., etoposide and teniposide, both of which are useful in the treatment of testicular cancer and Kaposi's sarcoma; the antibiotic chemotherapeutic agents, e.g., daunorubicin, doxorubicin,
20 epirubicin, mitomycin (used to treat stomach, cervix, colon, breast, bladder and pancreatic cancer), dactinomycin, temozolomide, plicamycin, bleomycin (used in the treatment of skin, esophagus and genitourinary tract cancer); and the enzymatic chemotherapeutic agents such as L-asparaginase.

25 In addition to the above, a compound, salt or prodrug of this invention might be expected to have a beneficial effect used in combination with the platinum coordination complexes (cisplatin, etc.); substituted ureas such as hydroxyurea; methylhydrazine derivatives, e.g., procarbazine; adrenocortical
30 suppressants, e.g., mitotane, aminoglutethimide; and hormone and hormone antagonists such as the adrenocorticosteroids (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate); estrogens (e.g., diethylstilbesterol); antiestrogens such as tamoxifen; androgens, e.g., testosterone propionate;
35 and aromatase inhibitors such as anastrozole.

The combination of a compound of this invention might be expected to be particularly effective in combination with mitoxantrone or paclitaxel for the treatment of solid tumor cancers or leukemias such as, without limitation, acute myelogenous (non-lymphocytic) leukemia.

Lastly, in addition to the above, a compound, salt or prodrug of this invention might be expected to have a beneficial effect used in combination with COX-2 inhibitor. To treat inflammation. COX-2 inhibitors for use in

combination with a compound, salt or prodrug of the preferred embodiments of the present invention might include, without limitation, those disclosed in WO 96/41626 and U.S. Patent No. 6,248,745. Other COX-2 inhibitors for use in the combinations of the invention include those disclosed in *Drugs of the Future*, 1997, 22, 711-714 which document is incorporated herein by reference, namely Meloxicam, L-745337 (Merck), MK-966 (Merck), L-768277 (Merck), GR-253035 (Glaxo-Wellcome), JTE-522 (Japan Tobacco), RS-57067-000 (Roche), SC-58125 (Searle), SC-078 (Searle), PD-138387 (Warner-Lambert), NS-398 (Taisho), flosulide and PD-164387 (Warner-Lambert).

EXAMPLES

The compounds of this invention may be readily synthesized using techniques well known in the chemical arts. It will be appreciated by those skilled in the art that other synthetic pathways for forming the compounds of the invention, and others like them, are available and that the following is offered by way of example and not limitation. In addition, such other synthetic pathways are within the scope of this invention.

The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

Synthetic Examples

Preparation of 4-heteroarylindolinones

4-Pyridin-4-yl-1,3-dihydroindol-2-one

5 Palladium catalyst $\text{PdCl}_2(\text{dppf}) \cdot \text{CH}_2\text{Cl}_2$ (1.22 g, 1.5 mmol) was added to a mixture of 4-bromoindole (9.80 g, 50 mmol), bis(pinacolato)diboron (13.97 g, 55 mmol), and potassium acetate (14.72 g, 150 mmol) in DMSO (200 mL). The system was degassed, and then purged three times with nitrogen. The
10 mixture was stirred at 80° C in an oil bath under nitrogen for 22 hours. It was then cooled to room temperature and poured into water (1 L). The aqueous mixture was extracted with three portions of ethyl acetate. The combined extracts were washed five times with brine to remove DMSO and then dried
15 over anhydrous Na_2SO_4 . The residue was purified on a silica gel column, eluting with EtOAc-hexane (9:1), to give 8.01g (66%) of 4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1H-indole.

^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 11.03 (br s, 1H, NH), 7.49 (d, $J = 7.7$ Hz, 1H), 7.38 (dd, $J = 0.9$ & 7.0 Hz, 1H), 7.38 (t, $J = 2.6$ Hz, 1H), 7.06 (dd, $J = 7.7$ & 7.0 Hz, 1H), 6.73 (br d, $J = 2.2$ Hz, 1H), 1.32 (s, 12H, 4 CH_3).

MS m/e 244 [$\text{M}^+ + 1$].

Palladium catalyst $\text{Pd}(\text{PPh}_3)_4$ (1.00g, 0.87 mmol) and freshly prepared aqueous sodium hydroxide (4.63 g, 115.9 mmol
25 in 42 mL water) were added to a mixture of 4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1H-indole (7.03g, 28.9 mmol) and 4-bromopyridine hydrochloride (5.68 g, 29.2 mmol) in THF (98 mL). The system was degassed and then purged three times with nitrogen. The mixture was stirred under nitrogen at
30 70° C in an oil bath for 6 hours. It was then cooled to room temperature and ethyl acetate (400 mL) added. The organic layer was isolated, washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was triturated with dichloromethane to give 5.1 g (91%) of 4-pyridin-4-yl-1H-

indole.

^1H NMR (300 MHz, DMSO- d_6) δ 11.39 (br s, 1H, NH), 8.65 (dd, J = 1.5 & 4.6 Hz, 2H), 7.67 (dd, J = 1.6 & 4.6 Hz, 2H), 7.49 (m, 2H), 7.20 (m, 2H), 6.62 (br d, J = 3.0 Hz, 1H).

5 Pyridinium tribromide (90% (Aldrich), 24.70 g, 69.50 mmol) was added portion-wise over 10 minutes to a suspension of 4-pyridin-4-yl-1H-indole (4.50 g, 23.17 mmol) in 2-methyl-2-propanol (135 mL), ethanol (90 mL) and acetic acid (45 mL).

10 The mixture was stirred at room temperature for one hour and then acetic acid (180 mL) was added. After stirring for an additional hour, water (1 mL) and zinc dust (15.06 g, 232 mmol) were added and stirring was continued for another hour.

15 Residual zinc dust was removed by filtration and washed with methanol. The filtrate was concentrated and the syrupy residue was stirred in water (500 mL) overnight. The solid that formed was filtered, washed with water to remove zinc and pyridine salts and dried under high vacuum dry to give 5.85 g (99%) of 4-pyridin-4-yl-1,3-dihydroindol-2-one as a light yellow solid.

20 ^1H NMR (300 MHz, DMSO- d_6) δ 10.54 (br s, 1H, N-H), 8.62 (d, J = 5.3 Hz, 2H), 7.59 (d, J = 5.3 Hz, 2H), 7.32 (dd, J = 8.3 & 7.4 Hz, 1H, H-6), 7.10 (d, J = 8.3 Hz, H-7), 6.89 (d, J = 7.4 Hz, H-5), 3.68 (s, 2H, CH_2).

MS m/e 211 [$\text{M}^+ + 1$].

25

4-Piperidin-4-yl-1,3-dihydroindol-2-one

30 To a suspension of 4-pyridin-4-yl-1,3-dihydroindol-2-one acetic acid salt (5.50 g, 20.4 mmol) in methanol (160 mL), water (70 mL) and acetic acid (30 mL) was added concentrated hydrochloric acid (2 mL) followed by platinum(IV) oxide (360 mg). The mixture was hydrogenated for three days. It was then filtered through celite, which was washed with methanol.

The filtrate was evaporated and dried under high vacuum. The residue was dissolved in methanol (500 mL) and treated with a

basic resin (hydroxide form) at pH = 9-10. The resin was removed by filtration and washed with methanol. The filtrate was evaporated and dried under high vacuum to give 4.2 g (96%) of 4-piperidin-4-yl-1,3-dihydroindol-2-one.

5 MS m/e 217 [M⁺+1].

4-(1-Methylpiperidin-4-yl)-1,3-dihydroindol-2-one

Methyl iodide (130.6 mg, 0.92 mmol) was added to a solution of 4-piperidin-4-yl-1,3-dihydroindol-2-one (199 mg, 0.92 mmol) in acetonitrile (10 mL) and methanol (1 mL). The reaction was stirred at room temperature under nitrogen for 2 days. The solvents were removed and the residue was purified on a silica gel column to give 150 mg (70%) of 4-(1-methylpiperidin-4-yl)-1,3-dihydroindol-2-one as a tan solid.

MS m/z 231 [M⁺+1].

4-Thiazol-2-yl-1,3-dihydroindol-2-one

Palladium catalyst Pd(PPh₃)₄ (142 mg, 0.12 mmol) and freshly prepared aqueous sodium hydroxide (493 mg, 12.33 mmol in 6 mL of water) were added to a mixture of 4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1H-indole (1 g, 4.11 mmol) and 2-bromothiazole (809 mg, 4.93 mmol) in THF (20 mL).

The system was degassed and then purged three times with nitrogen. The mixture was stirred under nitrogen at 70° C in an oil bath for 4 hours. It was then cooled to room temperature and ethyl acetate (300 mL) was added. The organic layer was isolated, washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The residue was triturated with dichloromethane to give 350 mg (42%) of 4-thiazol-2-yl-1H-indole as a pale yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ 11.45 (br s, 1H, NH), 7.99 (d, J = 3.3 Hz, 1H), 7.79 (d, J = 3.3 Hz, 1H), 7.69 (dd, J =

1 & 7.2 Hz, 1H), 7.53 (m, 2H), 7.21 (t, $J = 7.7$ Hz, 1H), 7.13 (m, 1H).

MS +ve APCI 201 [$M^+ + 1$].

Pyridinium tribromide (90% (Aldrich), 1.9 g, 5.34 mmol) was added portion-wise over 10 minutes to a suspension of 4-thiazol-2-yl-1H-indole (360 mg, 1.78 mmol) in 2-methyl-2-propanol (15 mL), ethanol (9 mL) and acetic acid (5 mL). The mixture was stirred at room temperature for 2 hours after which acetic acid (18 mL), water (1 mL) and zinc dust (1.5 g, 23.14 mmol) were added. Stirring was continued for 1.5 hours.

Residual zinc dust was removed by filtration and washed with methanol. The filtrate was concentrated and the syrupy residue was stirred in water (50 mL) overnight. The solid which formed was filtered, washed with water to remove the zinc and pyridine salts and dried under high vacuum to give 4-thiazol-2-yl-1,3-dihydroindol-2-one.

^1H NMR (400 MHz, DMSO- d_6) δ 10.58 (br s, 1H, NH), 7.99 (d, $J = 3.2$ Hz, 1H), 7.86 (d, $J = 3.2$ Hz, 1H), 7.56 (dd, $J = 0.9$ & 7.8 Hz, 1H), 7.32 (t, $J = 7.8$ Hz, 1H), 6.92 (d, $J = 7.8$ Hz, 1H), 3.75 (s, 2H, CH_2).

MS +ve APCI m/z 217 [$M^+ + 1$].

4-Pyrimidin-5-yl-1,3-dihydroindol-2-one

Palladium catalyst $\text{Pd}(\text{PPh}_3)_4$ (142 mg, 0.12 mmol) and freshly prepared aqueous sodium hydroxide (493 mg, 12.33 mmol, in 6 mL of water) were added to a mixture of 4-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)-1H-indole (1 g, 4.11 mmol) and 5-bromopyrimidine (784 mg, 4.93 mmol) in THF (20 mL). The system was degassed and then purged three times with nitrogen. The mixture was stirred under nitrogen at 70°C in an oil bath for 4 hours. It was then cooled to room temperature and ethyl acetate (300 mL) was added. The organic layer was isolated, washed by brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was triturated with dichloromethane

to give 570 mg (71%) of 4-pyrimidin-5-yl-1*H*-indole as a pale green solid.

¹H NMR (400 MHz, DMSO-*d*₆) δ 11.44 (br s, 1H, NH), 9.21 (s, 1H), 9.11 (s, 2H), 7.52 (m, 2H), 7.24 (m, 2H), 6.60 (m, 1H).

MS *m/z* 196 [*M*⁺+1].

Pyridinium tribromide (90% (Aldrich), 4 g, 11.22 mmol) was added portion-wise over 10 minutes to a suspension of 4-pyrimidin-5-yl-1*H*-indole (730 mg, 3.74 mmol) in 2-methyl-2-propanol (25 mL), ethanol (15 mL) and acetic acid (9 mL). The mixture was stirred at room temperature for 2 hours after which acetic acid (36 mL), water (1 mL) and zinc (3.2 g, 56.1 mmol) were added. Stirring was continued for 1.5 hours.

Residual zinc dust was filtered and washed with methanol. The filtrate was concentrated and the syrupy residue was stirred in water (100 mL) overnight. The precipitate which formed was filtered, washed with water to remove zinc and pyridine salts and dried under high vacuum to give 595 mg (75%) of 4-pyrimidin-5-yl-1,3-dihydroindol-2-one as a light brown solid.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.59 (d, *J* = 3.8 Hz, 2H), 7.84 (m, 1H), 7.44 (m, 2H), 3.35 (s, 2H, CH₂).

MS -ve APCI *m/z* 210 [*M*⁺-1].

4-(6-Aminopyridin-3-yl)-1,3-dihydroindol-2-one

Palladium catalyst Pd(PPh₃)₄ (142 mg, 0.12 mmol) and freshly prepared aqueous sodium hydroxide (493 mg, 12.33 mmol in 6 mL of water) were added to a mixture of 4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1*H*-indole (1 g, 4.11 mmol) and 2-amino-5-bromopyridine (853 mg, 4.93 mmol) in THF (20 mL). The system was degassed and then purged three times with nitrogen. The mixture was stirred under nitrogen at 80° C in an oil bath for 4.5 hours. It was then cooled to room temperature and ethyl acetate (300 mL) was added. The organic layer was isolated, washed with brine, dried over anhydrous

Na₂SO₄ and concentrated. The residue was purified by column chromatography to give 720 mg (84%) of 5-(1*H*-indol-4-yl)-pyridin-2-ylamine as a thick brown syrup.

¹H NMR (400 MHz, DMSO-*d*₆) δ 11.18 (br s, 1H, NH), 8.20
5 (d, *J* = 0.9 Hz, 1H), 7.67 (dd, *J* = 2.4 & 8.5 Hz, 1H), 7.36
(t, *J* = 2.8 Hz, 1H), 7.32 (d, *J* = 7.3 Hz, 1H), 7.11 (t, *J* =
7.7 Hz, 1H), 6.97 (d, *J* = 6.5 Hz, 1H), 6.56 (dd, *J* = 0.6 &
7.9 Hz, 1H), 6.50 (m, 1H), 5.99 (s, 2H, NH₂).

MS -APCI *m/z* 208 [M⁺-1].

10 Pyridinium tribromide (90% (Aldrich), 3.7 g, 10.32 mmol)
was added portion-wise over 10 minutes to a suspension of 5-
(1*H*-indol-4-yl)-pyridin-2-ylamine (720 mg, 3.44 mmol) in 2-
methyl-2-propanol (25 mL), ethanol (15 mL) and acetic acid (9
15 mL). The mixture was stirred at room temperature for 2 hours
and then acetic acid (36 mL), water (1 mL) and zinc dust (3.2
g, 56.1 mmol) were added. Stirring was continued for 1.5
hours. Residual zinc dust was filtered and washed with
methanol. The filtrate was concentrated and the syrupy
residue was stirred in water (100 mL) overnight. The solid
20 that formed was filtered, washed with water to remove zinc and
pyridine salts and dried under high vacuum to give 750 mg
(97%) of 4-(6-amino-pyridin-3-yl)-1,3-dihydroindol-2-one as a
light brown solid.

MS +ve APCI *m/z* 226 [M⁺+1].

25

4-Pyridin-2-yl-1,3-dihydroindol-2-one

Palladium catalyst Pd(PPh₃)₄ (714 mg, 0.6 mmol) and
freshly prepared aqueous sodium hydroxide (2.47 g, 61.8 mmol
in 22 mL of water) were added to a mixture of 4-(4,4,5,5-
30 tetramethyl-[1,3,2]dioxaborolan-2-yl)-1*H*-indole (5 g, 20.6
mmol) and 2-bromopyridine (2 mL, 20.8 mmol) in THF (70 mL).
The system was degassed and then purged three times with
nitrogen. The mixture was stirred under nitrogen at 70°C in
an oil bath for 6 hours. It was then cooled to room

temperature and 400 ml ethyl acetate was added. The organic layer was isolated, washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The residue was triturated with dichloromethane to give 2.85 g (71%) of 4-pyridin-2-yl-1H-indole as a pale yellow solid.

¹H NMR (360 MHz, DMSO-*d*₆) δ 11.27 (br s, 1H, NH), 8.72 (d, *J* = 4.6 Hz, 1H), 7.89 (m, 2H), 7.52 (d, *J* = 7.7 Hz, 1H), 7.49 (d, *J* = 7.7 Hz, 1H), 7.44 (t, *J* = 2.8 Hz, 1H), 7.33 (m, 1H), 7.21 (t, *J* = 7.7 Hz, 1H), 6.93 (m, 1H).

MS +APCI *m/z* 195 [*M*⁺+1].

Pyridinium tribromide (90% (Aldrich), 15.4 g, 43.2 mmol) was added portion-wise over 10 minutes to a suspension of 4-pyridin-2-yl-1H-indole (2.8 g, 14.4 mmol) in 2-methyl-2-propanol (84 mL), ethanol (56 mL) and acetic acid (28 mL).

The mixture was stirred at room temperature for 2 hours after which acetic acid (100 mL) was added. Stirring was continued for one hour and then water (0.5 mL) and zinc dust (9.4 g, 144 mmol) were added. Stirring was continued for another hour. Residual zinc dust was filtered and washed with methanol. The filtrate was concentrated and the syrupy residue was stirred in water (300 mL) overnight. The solid that formed was filtered, washed with water to remove zinc and pyridine salts and dried under high vacuum to give 2.8 g (92%) of 4-pyridin-2-yl-1,3-dihydroindol-2-one was obtained.

¹H NMR (400 MHz, DMSO-*d*₆) δ 10.49 (s, 1H, NH), 8.68 (d, *J* = 4.2 Hz, 1H), 7.83-7.91 (m, 2H), 7.45 (d, *J* = 7.3 Hz, 1H), 7.29-7.38 (m, 2H), 6.89 (d, *J* = 7.4 Hz, 1H), 3.77 (s, 2H, CH₂).

MS +ve APCI *m/z* 211 [*M*⁺+1].

4-Pyridin-3-yl-1,3-dihydroindol-2-one

Palladium catalyst Pd(PPh₃)₄ (284 mg, 0.25 mmol) and freshly prepared aqueous sodium hydroxide (984 mg in 9 mL of water) were added to a mixture of 4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1H-indole (2 g, 8.2 mmol) and 3-bromopyridine (0.8 mL, 8.3 mmol) in THF (28 mL). The system

was degassed and then purged three times with nitrogen. The mixture was stirred under nitrogen at 70° C in an oil bath for 6 hours. It was then cooled to room temperature and 400 ml ethyl acetate was added. The organic layer was isolated, washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The residue was column chromatographed to give 1 g (62%) of 4-pyridin-3-yl-1H-indole as a white solid.

¹H NMR (360 MHz, DMSO-*d*₆) δ 11.32 (br s, 1H, NH), 8.87 (d, *J* = 2.1 Hz, 1H), 8.58 (dd, *J* = 2.1 & 4.8 Hz, 1H), 8.06 (dt, *J* = 2.1 & 8.1 Hz, 1H), 7.52 (dd, 1H), 7.46 (m, 2H), 7.22 (t, *J* = 7.6 Hz, 1H), 7.14 (t, *J* = 7.1 Hz, 1H), 6.54 (d, *J* = 3.2 Hz, 1H).

MS +ve APCI *m/z* 195 [M⁺+1].

Pyridinium tribromide (90% (Aldrich), 5.5 g, 15.3 mmol) was added portion-wise over 10 minutes to a suspension of 4-pyridin-3-yl-1H-indole (1 g, 5.1 mmol) in 2-methyl-2-propanol (30 mL), ethanol (20 mL) and acetic acid (10 mL). The mixture was stirred at room temperature for 2 hours and then acetic acid (50 mL) was added. After stirring for an additional hour, water (0.5 mL) and zinc dust (3.3 g, 51 mmol) were added and stirring was continued for another hour. Residual zinc dust was filtered and washed with methanol. The filtrate was concentrated and the syrupy residue was stirred in water (100 mL) overnight. The solid which formed was filtered, washed with water to remove zinc and pyridine salts and dried under high vacuum to give 1.1 g (100%) of 4-pyridin-3-yl-1,3-dihydro-indol-2-one.

¹H NMR (400 MHz, DMSO-*d*₆) δ 10.52 (br s, 1H, NH), 8.78 (d, *J* = 1.8 Hz, 1H), 8.58 (dd, *J* = 1.5 & 4.7 Hz, 1H), 8.02 (m, 1H), 7.5 (m, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.06 (d, *J* = 7.0 Hz, 1H), 6.88 (d, *J* = 7.6 Hz, 1H), 3.64 (s, 2H, CH₂).

MS +ve APCI *m/z* 211 [M⁺+1].

5-(2-Oxo-2,3-dihydro-1H-indol-4-yl)-nicotinic acid

Palladium catalyst Pd(PPh₃)₄ (693 mg, 0.6 mmol) and freshly prepared aqueous sodium hydroxide (3.2 g in 29 mL of water) were added to a mixture of 4-(4,4,5,5-tetramethyl[1,3,2]-dioxaborolan-2-yl)-1H-indole (4.9 g, 20.2 mmol) and 5-bromonicotinic acid (4.04 g, 20 mmol) in THF (70 mL). The system was degassed and then purged three times with nitrogen. The mixture was refluxed under nitrogen for 6 hours. It was then cooled to room temperature and ethyl acetate (400 mL) added. The organic layer was separated and washed twice with 2N NaOH solution. The aqueous layer and hydroxide washes were combined, washed with dichloromethane and then acidified with 6N HCl. The resulting precipitate was collected by vacuum filtration, washed with water and dried to give 2.3 g (48%) 5-(1H-indol-4-yl)-nicotinic acid.

¹H NMR (360 MHz, DMSO-*d*₆) δ 11.45 (br s, 1H, NH), 9.06 (m, 2H), 8.49 (m, 1H), 7.45 (m, 2H), 7.22 (m, 2H), 6.54 (s, 1H).

MS +ve APCI *m/z* 239 [M⁺+1].

Pyridinium tribromide (90% (Aldrich), 9.8 g, 27.6 mmol) was added portion-wise over 10 minutes to a suspension of 5-(1H-indol-4-yl)-nicotinic acid (2.2 g, 9.2 mmol) in 2-methyl-2-propanol (54 mL), ethanol (36 mL) and acetic acid (108 mL). The mixture was stirred at room temperature for 2 hours and then acetic acid (10 mL) was added. After stirring for an additional hour, water (0.5 mL) and zinc dust (6 g, 92 mmol) were added and stirring was continued for another hour. Residual zinc dust was filtered and washed with methanol. The filtrate was concentrated and the syrupy residue was stirred in water (300 mL) overnight. The solid which formed was filtered, washed with water to remove zinc and pyridine salts and dried under high vacuum to give 2.3 g (98%) of 5-(2-oxo-2,3-dihydro-1H-indol-4-yl)-nicotinic acid.

¹H NMR (400 MHz, DMSO-*d*₆) δ 10.53 (s, 1H, NH), 9.08 (s, 1H), 8.92 (s, 1H), 8.35 (s, 1H), 7.32 (t, *J* = 7.5 Hz, 1H),

7.09 (d, $J = 7.5$ Hz, 1H), 6.89 (d, $J = 7.5$ Hz, 1H), 3.65 (s, 2H, CH₂).

MS -ve APCI m/z 253 [$M^+ - 1$].

5 4-(2-amino-pyrimidin-5-yl)-1,3-dihydro-indol-2-one

Pd(PPh₃)₄ (285 mg, 0.25 mmol) and a freshly prepared sodium hydroxide solution (985 mg in 12 mL of water) were added to a mixture of 4-(4,4,5,5-

10 tetramethyl[1,3,2]dioxaborolan-2-yl)-1H-indole (2 g, 8.21 mmol) and 2-amino-5-bromopyrimidine (1.71 g, 9.85 mmol) in THF (30 mL). The reaction mixture was degassed and then purged three times with nitrogen and then refluxed overnight with stirring under nitrogen. The reaction mixture was cooled to
15 room temperature and then diluted with ethyl acetate (300 mL).

The organic layer was separated, washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The residue was purified by column chromatography to give 1.4 g (82%) of 5-(1H-indol-4-yl)-pyrimidin-2-ylamine as a white solid.

20 MS +ve APCI m/z 211 [$M^+ + 1$].

Pyridinium tribromide (90% purity from Aldrich, 7.1 g, 19.98 mmol) was added in portions over 10 minutes to a suspension of 5-(1H-indol-4-yl)-pyrimidin-2-ylamine (1.4 g, 6.66 mmol) in 2-methyl-2-propanol (50 mL), ethanol (30 mL) and
25 acetic acid (18 mL). The mixture was stirred at room temperature for 2 hours, and then acetic acid (72 mL), water (1 mL) and zinc (6.5 g, 100 mmol) were added. Stirring was continued for 1.5 hours. The unreacted zinc dust was filtered off and washed with methanol. The filtrate was concentrated
30 and the syrupy residue was suspended in water (100 mL) overnight. The solid which formed was filtered off. The filtrate was basified with aqueous sodium bicarbonate and extracted with ethanol:dichloromethane (5:95) to give 4-(2-amino-pyrimidin-5-yl)-1,3-dihydro-indol-2-one.

35 ¹H NMR (360 MHz, DMSO-*d*₆) δ 10.46 (s, 1H, NH), 8.47 (s,

2H), 7.24 (t, $J = 7.6$ Hz, 1H), 6.99 (d, $J = 7.6$ Hz, 1H), 6.79 (d, $J = 7.6$ Hz, 1H), 6.78 (s, 2H, NH₂), 3.64 (s, 2H).

MS +ve APCI m/z 227 [$M^+ + 1$].

5

General amidation procedure

A mixture of a carboxylic acid pyrrole aldehyde (1 equiv.), an appropriately substituted amine (1.2 equiv.), 1-hydroxybenzotriazole (HOBt, 1.2 equiv.) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC, 1.2 equiv.) were dissolved in sufficient N,N-dimethylformamide (DMF) to make a 0.4 M solution. The mixture is stirred overnight at room temperature and then diluted with dichloromethane, washed with aqueous sodium bicarbonate, dried and concentrated to give the desired amide.

15 Utilizing the 4-heteroarylindolinones described above and following the amidation procedures following compounds of Formula (I) were prepared.

Example 1

20 3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-pyridin-4-yl-1,3-dihydroindol-2-one

A mixture of 4-pyridin-4-yl-1,3-dihydroindol-2-one (50 mg, 0.24 mmol), 3,5-dimethyl-4-(4-methyl-piperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (59 mg, 0.24 mmol) and piperidine (0.1 mL) in ethanol (1 mL) was heated at 60° C for 5 hours. The reaction was concentrated and the residue was column chromatographed to give the title compound.

¹H NMR (300 MHz, DMSO-*d*₆) δ 13.42 (br s, 1H, NH), 11.09 (br s, 1H, NH), 8.72 (d, $J = 5.5$ Hz, 2H), 7.49 (d, $J = 5.5$ Hz, 2H), 7.21 (t, $J = 7.7$ Hz, 1H), 6.96 (d, $J = 7.7$ Hz, 1H), 6.79 (d, $J = 7.7$ Hz, 1H), 6.72 (s, 1H, H-vinyl), 3.35 (m, 4H, 2xCH₂), 2.26 (m, 4H, 2xCH₂), 2.23 (s, 3H, CH₃), 2.18 (s, 3H, CH₃), 1.54 (s, 3H, CH₃).

MS m/z 442 [$M^+ + 1$].

Example 2

3-(5-Methyl-3H-imidazol-4-ylmethylene)-4-pyridin-4-yl-1,3-dihydroindol-2-one

A mixture of 4-pyridin-4-yl-1,3-dihydroindol-2-one (50 mg, 0.24 mmol), 5-methyl-3H-imidazole-4-carbaldehyde (24.4 mg, 0.24 mmol) and piperidine (1 drop) in ethanol (2 mL) was stirred at room temperature for 2 days. The precipitate which formed was filtered. Crystals which formed in the filtrate were isolated, washed with ethanol and dried to give 7.8 mg of the title compound.

^1H NMR (360 MHz, DMSO- d_6) δ 13.55 (br s, 1H, NH), 11.13 (br s, 1H, NH), 8.75 (d, J = 6.0 Hz, 2H), 7.85 (s, 1H), 7.50 (d, J = 6.0 Hz, 2H), 7.27 (t, J = 7.7 Hz, 1H), 6.97 (d, J = 7.7 Hz, 1H), 6.83 (d, J = 7.7 Hz, 1H), 6.76 (s, 1H, H-vinyl), 1.78 (m, 3H, CH_3).

MS m/z 303 [$M^+ + 1$].

Example 3

1-(2-Oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-6,7-dihydro-2H-pyrano[3,4-c]pyrrol-4-one

A mixture of 4-pyridin-4-yl-1,3-dihydroindol-2-one (50 mg, 0.24 mmol), 4-oxo-2,4,6,7-tetrahydropyrano[3,4-c]pyrrole-1-carbaldehyde (39.2 mg, 0.24 mmol) and piperidine (1 drop) in ethanol (2 mL) was stirred at room temperature for 48 hours. The precipitate which formed was collected by vacuum filtration, washed with ethanol and dried to give 15 mg (17%) of the title compound.

^1H NMR (360 MHz, DMSO- d_6) δ 8.74 (d, J = 6.1 Hz, 2H), 7.83 (s, 1H), 7.48 (d, J = 6.1 Hz, 2H), 7.28 (t, J = 7.7 Hz, 1H), 6.99 (d, J = 7.7 Hz, 1H), 6.84 (d, J = 7.7 Hz, 1H), 6.66 (s, 1H), 4.34 (t, J = 5.7 Hz, 2H, CH_2), 2.29 (t, J = 5.7 Hz, 2H,

CH₂).

MS *m/z* 358 [M⁺+1].

Example 4

5 3-[3-Methyl-4-(piperidine-1-carbonyl)-1*H*-pyrrol-2-ylmethylene]-4-pyridin-4-yl-1,3-dihydroindol-2-one

10 A mixture of 4-pyridin-4-yl-1,3-dihydroindol-2-one (60 mg, 0.3 mmol), 3-methyl-4-(piperidine-1-carbonyl)-1*H*-pyrrole-2-carbaldehyde (75 mg, 0.34 mmol) and piperidine (0.14 mL) in ethanol (2 mL) was stirred at 100° C overnight. The reaction was column chromatographed (2-3% methanol in dichloromethane) to give 20 mg (17%) of the title compound as a yellow orange solid.

15 ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.49 (br s, 1H, NH), 11.16 (br s, 1H, NH), 8.73 (d, *J* = 6.1 Hz, 2H), 7.50 (d, *J* = 6.1 Hz, 2H), 7.37 (d, *J* = 3.1 Hz, 1H), 7.24 (t, 1H), 6.96 (d, 1H), 6.80 (d, 1H), 6.79 (s, 1H, H-vinyl), 3.40 (m 4H, 2xCH₂), 1.6 (s, 3H, CH₃), 1.56 (m, 2H, CH₂), 1.44 (m, 4H, 2xCH₂).

20 MS *m/z* 413 [M⁺+1].

Example 5

3-(3,5-Dimethyl-1*H*-pyrrol-2-ylmethylene)-4-pyridin-4-yl-1,3-dihydroindol-2-one

25 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 3,5-dimethyl-1*H*-pyrrole-2-carbaldehyde to give the title compound.

30 ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.22 (br s, 1H, NH), 10.99 (br s, 1H, NH), 8.73 (d, *J* = 5.7 Hz, 2H), 7.48 (d, *J* = 5.7 Hz, 2H), 7.18 (t, *J* = 7.6 Hz, 1H), 6.96 (d, *J* = 7.6 Hz, 1H), 6.78 (d, *J* = 7.6 Hz, 1H), 6.72 (s, 1H, H-vinyl), 5.92 (s, 1H), 2.27 (s, 3H, CH₃), 1.61 (s, 3H, CH₃).

MS +ve APCI *m/z* 316 [M⁺+1].

Example 6

3-[2-(2-Oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid

5 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid to give the title compound.

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.24 (br s, 1H, NH), 12.0 (br s, 1H, COOH), 10.98 (br s, 1H, NH), 8.68 (d, *J* = 5.9 Hz, 2H),
10 7.48 (d, *J* = 5.9 Hz, 2H), 7.17 (t, *J* = 7.8 Hz, 1H), 6.95 (d, *J* = 7.8 Hz, 1H), 6.84 (s, 1H, H-vinyl), 6.75 (d, *J* = 7.8 Hz, 1H), 2.63 (t, 2H, CH₂), 2.36 (t, 2H, CH₂), 2.16 (t, 2H, CH₂), 2.01 (t, 2H, CH₂), 1.64-1.73 (m, 4H).

MS +ve APCI *m/z* 414 [M⁺+1].

15

Example 7

3-[5-Methyl-2-(2-oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid

20 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 3-(2-formyl-5-methyl-1H-pyrrol-3-yl)-propionic acid to give the title compound.

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.31 (br s, 1H, NH), 12.0 (br s, 1H, COOH), 11.02 (br s, 1H, NH), 8.70 (d, *J* = 5.8 Hz, 2H),
25 7.48 (d, *J* = 5.8 Hz, 2H), 7.19 (t, *J* = 8 Hz, 1H), 6.96 (d, *J* = 8 Hz, 1H), 6.82 (s, 1H, H-vinyl), 6.78 (d, *J* = 8 Hz, 1H), 5.96 (d, *J* = 2.8 Hz, 2H), 2.28 (s, 3H, CH₃), 2.17 (m, 4H).

MS +ve APCI *m/z* 374 [M⁺+1].

Example 8

3-[5-Ethyl-2-(2-oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid

5 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with
3-(5-ethyl-2-formyl-1H-pyrrol-3-yl)-propionic acid to give the
title compound.

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.39 (br s, 1H, NH), 10.98
(br s, 1H, NH), 8.73 (d, *J* = 5.2 Hz, 2H), 7.56 (d, *J* = 5.2 Hz,
10 2H), 7.18 (t, *J* = 7.7 Hz, 1H), 6.97 (d, *J* = 7.7 Hz, 1H), 6.79
(s, 1H, H-vinyl), 6.78 (d, *J* = 7.7 Hz, 1H), 6.01 (d, *J* = 1.9
Hz, 1H), 2.63 (q, *J* = 7.7 Hz, 2H, CH₂CH₃), 2.16 (m, 4H), 1.18
(t, *J* = 7.7 Hz, 3H, CH₂CH₃).

MS +ve APCI *m/z* 388 [M⁺+1].

Example 9

*4-(2-Carboxyethyl)-2-methyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid
ethyl ester*

20 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with
4-(2-carboxyethyl)-5-formyl-2-methyl-1H-pyrrole-3-carboxylic
acid ethyl ester to give the title compound.

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.82 (br s, 1H, NH), 11.8 (br
25 s, 1H, COOH), 11.21 (br s, 1H, NH), 8.68 (d, *J* = 5.5 Hz, 2H),
7.49 (d, *J* = 5.5 Hz, 2H), 7.25 (t, *J* = 7.6 Hz, 1H), 6.98 (d, *J*
= 7.6 Hz, 1H), 6.93 (s, 1H, H-vinyl), 6.80 (d, *J* = 7.6 Hz,
1H), 4.16 (q, *J* = 7.2 Hz, 2H, OCH₂CH₃), 2.41 (t, *J* = 7.4 Hz,
2H), 2.14 (t, *J* = 7.4 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H,
30 OCH₂CH₃).

MS +ve APCI *m/z* 446 [M⁺+1].

Example 10

3-[2,4-Dimethyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid

5 4-Pyridin-4-yl-1,3-dihydro-indol-2-one was condensed with 3-(5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)-propionic acid to give the title compound.

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.28 (br s, 1H, NH), 12.0 (br s, 1H, COOH), 10.96 (br s, 1H, NH), 8.73 (d, *J* = 6 Hz, 2H),
10 7.48 (d, *J* = 6 Hz, 2H), 7.17 (t, *J* = 7.6 Hz, 1H), 6.95 (d, *J* = 7.6 Hz, 1H), 6.77 (d, *J* = 7.6 Hz, 1H), 6.72 (s, 1H, H-vinyl), 2.54 (t, *J* = 7.6 Hz, 2H), 2.26 (m, 5H), 1.55 (s, 3H, CH₃).

MS +ve APCI *m/z* 388 [M⁺+1].

15 **Example 11**

[2,4-Dimethyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-acetic acid

20 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with (5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)-acetic acid to give the title compound.

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.31 (s, 1H, NH), 12.0 (br s, 1H, COOH), 10.95 (s, 1H, NH), 8.72 (d, *J* = 5.9 Hz, 2H), 7.51 (d, *J* = 5.9 Hz, 2H), 7.17 (t, *J* = 7.7 Hz, 1H), 6.96 (d, *J* = 7.7 Hz, 1H), 6.77 (d, *J* = 7.6 Hz, 1H), 6.73 (s, 1H, H-vinyl),
25 3.18 (s, 2H, CH₂), 2.25 (s, 3H, CH₃), 1.54 (s, 3H, CH₃).

MS +ve APCI *m/z* 374 [M⁺+1].

Example 12

30 *3-(1H-Indol-2-ylmethylene)-4-pyridin-4-yl-1,3-dihydroindol-2-one*

4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 1H-indole-2-carbaldehyde to give the title compound.

¹H NMR (400 MHz, DMSO-*d*₆) δ 12.90 (br s, 1H, NH), 11.20

(br s, 1H, NH), 8.77 (d, $J = 5.5$ Hz, 2H), 7.56 (m, 4H), 7.33 (t, $J = 7.7$ Hz, 1H), 7.25 (m, 1H), 7.06 (m, 1H), 7.0 (d, $J = 7.7$ Hz, 1H), 6.90 (s, 1H, H-vinyl), 6.86 (d, $J = 7.7$ Hz, 1H), 6.56 (s, 1H).

5 MS +ve APCI m/z 338 [$M^+ + 1$].

Example 13

4-Pyridin-4-yl-3-(4,5,6,7-tetrahydro-1H-indol-2-ylmethylene)-
1,3-dihydroindol-2-one

10

4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 4,5,6,7-tetrahydro-1H-indole-2-carbaldehyde to give the title compound.

15

Example 14

3-[5-(2-Morpholin-4-yl-ethoxy)-1H-indol-2-ylmethylene]-4-pyridin-4-yl-1,3-dihydroindol-2-one

20 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with (5-(2-morpholin-4-yl-ethoxy)-1H-indole-2-carbaldehyde to give the title compound.

^1H NMR (400 MHz, DMSO- d_6) δ 12.82 (s, 1H, NH), 11.24 (s, 1H, NH), 8.77 (d, $J = 6.3$ Hz, 2H), 7.55 (d, $J = 6.3$ Hz, 2H), 7.48 (d, $J = 8.8$ Hz, 1H), 7.31 (t, $J = 7.7$ Hz, 1H), 7.03 (d, $J = 2.5$ Hz, 1H), 7.0 (d, $J = 7.7$ Hz, 1H), 6.91 (dd, $J = 2.5$ & 8.8 Hz, 1H), 6.84 (m, 2H), 6.43 (s, 1H), 4.05 (t, $J = 5.8$ Hz, 2H), 3.57 (t, $J = 4.6$ Hz, 4H), 2.68 (t, $J = 5.8$ Hz, 2H), 2.47 (br t, 4H).

25

MS +ve APCI m/z 467 [$M^+ + 1$].

30

Example 15

4-Methyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid

5 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 5-formyl-4-methyl-1H-pyrrole-2-carboxylic acid to give the title compound.

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.67 (br s, 1H, NH), 12.90 (br s, 1H, COOH), 11.22 (br s, 1H, NH), 8.75 (d, *J* = 5.8 Hz, 2H), 7.52 (d, *J* = 5.8 Hz, 2H), 7.30 (t, *J* = 7.7 Hz, 1H), 7.0 (d, *J* = 7.7 Hz, 1H), 6.78 (s, 1H, H-vinyl), 6.61 (d, *J* = 2.5 Hz, 1H), 1.64 (s, 3H, CH₃).

MS -ve APCI *m/z* 344 [M⁺-1].

Example 16

5-Methyl-2-(2-oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid

15 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 2-formyl-5-methyl-1H-pyrrole-3-carboxylic acid to give the title compound.

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.97 (s, 1H, NH), 12.08 (v br s, 1H, COOH), 11.26 (br s, 1H, NH), 8.63 (d, *J* = 6 Hz, 2H), 8.02 (s, 1H, H-vinyl), 7.42 (d, *J* = 6 Hz, 2H), 7.26 (t, *J* = 7.7 Hz, 1H), 6.98 (d, *J* = 7.7 Hz, 1H), 6.78 (d, *J* = 7.6 Hz, 1H), 6.39 (d, *J* = 2.3 Hz, 1H), 2.31 (s, 3H, CH₃).

MS -ve APCI *m/z* 344 [M⁺-1].

Example 17

30 3-[3-(3-Morpholin-4-yl-propyl)-4,5,6,7-tetrahydro-1H-indol-2-ylmethylene]-4-pyridin-4-yl-1,3-dihydroindol-2-one

4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 3-(3-morpholin-4-yl-propyl)-4,5,6,7-tetrahydro-1H-indole-2-carbaldehyde to give the title compound.

35 ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.22 (s, 1H, NH), 10.97 (s,

1H, NH), 8.72 (d, $J = 5.8$ Hz, 2H), 7.50 (d, $J = 5.9$ Hz, 2H),
7.17 (t, $J = 7.7$ Hz, 1H), 6.95 (d, $J = 7.7$ Hz, 1H), 6.81 (s,
1H, H-vinyl), 6.75 (d, $J = 7.7$ Hz, 1H), 3.55 (m, 4H), 2.64 (t,
2H), 2.34 (t, 2H), 2.29 (br s, 4H), 2.04 (t, 2H), 1.90 (t,
5 2H), 1.65-1.73 (m, 4H), 1.22 (m, 2H).

MS +ve APCI m/z 469 [$M^+ + 1$].

Example 18

10 *2,4-Dimethyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidene-methyl)-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide*

15 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)-amide to give the title compound.

^1H NMR (400 MHz, DMSO- d_6) δ 13.46 (s, 1H, NH), 11.10 (br s, 1H, NH), 8.74 (d, $J = 5.9$ Hz, 2H), 7.50 (d, $J = 5.9$ Hz, 2H), 7.35 (t, 1H, CONH), 7.22 (t, $J = 7.7$ Hz, 1H), 6.98 (d, $J = 7.7$ Hz, 1H), 6.81 (d, $J = 7.6$ Hz, 1H), 6.75 (s, 1H, H-
20 vinyl), 3.23 (q, $J = 6.8$ Hz, 2H, NCH_2CH_3), 2.45-2.5 (m, 6H), 2.39 (s, 3H, CH_3), 1.69 (s, 3H, CH_3), 0.95 (t, $J = 6.8$ Hz, 6H, $\text{N}(\text{CH}_2\text{CH}_3)_2$).

MS -ve APCI m/z 456 [$M^+ - 1$].

Example 19

25 *3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-pyridin-4-yl-1,3-dihydroindol-2-one*

30 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde to give the title compound.

MS -ve APCI m/z 440 [$M^+ - 1$].

Example 20

3-[3-Methyl-5-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-2-ylmethylen]-4-pyridin-4-yl-1,3-dihydroindol-2-one

5 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 3-methyl-5-(4-methylpiperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde to give the title compound.

Example 21

10 *3-(2-Oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid ethyl ester*

4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 3-formyl-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid ethyl ester to give the title compound.

15

Example 22

2-Methyl-5-(2-oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidene-methyl)-4-phenyl-1H-pyrrole-3-carboxylic acid pyridin-4-ylamide

20

4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 5-formyl-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid pyridin-4-ylamide to give the title compound.

25

Example 23

3-(5-Methylthiophen-2-ylmethylen)-4-pyridin-4-yl-1,3-dihydro-indol-2-one

30 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 5-methylthiophene-2-carbaldehyde to give the title compound.

Example 24

3-(4-Morpholin-4-yl-benzylidene)-4-pyridin-4-yl-1,3-dihydro-indol-2-one

5 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with
4-morpholin-4-yl-benzaldehyde to give the title compound.

Example 25

10 *4-[4-(2-Oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-
phenyl]-piperazine-1-carbaldehyde*

15 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with
4-(4-formylphenyl)-piperazine-1-carbaldehyde to give the title
compound.

Example 26

20 *4-(2-Carboxyethyl)-3-methyl-5-(2-oxo-4-pyridin-4-yl-1,2-
dihydro-indol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid
ethyl ester*

25 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with
4-(2-carboxyethyl)-5-formyl-3-methyl-1H-pyrrole-2-carboxylic
acid ethyl ester to give the title compound.

Example 27

30 *4-(2-Hydroxyethyl)-5-(2-oxo-4-pyridin-4-yl-1,2-dihydroindol-3-
ylidenemethyl)-1H-pyrrole-3-carboxylic acid*

35 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with
5-formyl-4-(2-hydroxyethyl)-1H-pyrrole-3-carboxylic acid to
give the title compound.

Example 28

4-(4-Methoxyphenyl)-5-(2-oxo-4-pyridin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

5 4-Pyridin-4-yl-1,3-dihydroindol-2-one was condensed with 5-formyl-4-(4-methoxyphenyl)-1H-pyrrole-3-carboxylic acid ethyl ester to give the title compound.

Example 29

10 3-(5-Methyl-3H-imidazol-4-ylmethylene)-4-piperidin-4-yl-1,3-dihydroindol-2-one, acetic acid salt

A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (124 mg, 0.57 mmol) and 5-methyl-3H-imidazole-4-carbaldehyde (62.8 mg, 0.57 mmol) in ethanol (3 mL) was stirred at room temperature for 2 days. The reaction was concentrated and the residue was column chromatographed (reverse phase) to give 23 mg of the title compound as a yellow acetic acid salt.

¹H NMR (300 MHz, DMSO-d₆) δ 13.89 (br s, 1H, NH), 11.02 (br s, 1H, NH), 7.90 (s, 1H), 7.52 (s, 1H), 7.15 (t, J = 7.8 Hz, 1H), 6.93 (d, J = 7.8 Hz, 1H), 6.76 (d, J = 7.8 Hz, 1H), 3.20 (m, 1H), 3.15 (m, 2H, CH₂), 2.73 (m, 2H, CH₂), 1.86 (m, 3H, CH₃), 1.84 (m, 2H, CH₂), 1.62 (m, 2H, CH₂).

MS m/z 309 [M⁺+1].

25

Example 30

3-[3-Methyl-4-(piperidine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-piperidin-4-yl-1,3-dihydro-indol-2-one

30 A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (86.5 mg, 0.4 mmol) and 3-methyl-4-(piperidine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (88.1 mg, 0.4 mmol) in ethanol (2 mL) was stirred at room temperature for 4 days. The reaction was concentrated and the residue was column chromatographed to give 52 mg (31%) of the title compound as a yellow solid.

35

¹H NMR (300 MHz, DMSO-*d*₆) δ 13.80 (br s, 1H, NH), 11.0 (br s, 1H, NH), 7.53 (s, 1H, H-vinyl), 7.40 (d, *J* = 3.0 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 6.93 (d, *J* = 7.8 Hz, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 3.48 (m, 4H, 2xCH₂), 3.20 (m, 1H), 3.10 (m, 2H, CH₂), 2.70 (m, 2H, CH₂), 2.29 (s, 3H, CH₃), 1.84 (m, 2H, CH₂), 1.61 (m, 4H, 2xCH₂), 1.48 (m, 4H, 2xCH₂).

MS *m/z* 419 [M⁺+1].

Example 31

10 3-[3-Methyl-4-(morpholine-4-carbonyl)-1H-pyrrol-2-ylmethylenel]-4-piperidin-4-yl-1,3-dihydroindol-2-one

A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (86.5 mg, 0.4 mmol) and 3-methyl-4-(morpholine-4-carbonyl)-1H-pyrrole-2-carbaldehyde (88.9 mg, 0.4 mmol) in ethanol (2 mL) was stirred at room temperature for 4 days. The reaction was concentrated and the residue was column chromatographed to give 54 mg (32%) of the title compound as a yellow solid.

¹H NMR (360 MHz, DMSO-*d*₆) δ 13.82 (br s, 1H, NH), 10.97 (br s, 1H, NH), 7.54 (s, 1H, H-vinyl), 7.45 (d, *J* = 3.0 Hz, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 6.94 (d, *J* = 7.8 Hz, 1H), 6.76 (d, *J* = 7.8 Hz, 1H), 3.58 (m, 4H, 2xCH₂), 3.53 (m, 4H, 2xCH₂), 3.20 (m, 1H), 3.12 (m, 2H, CH₂), 2.73 (m, 2H, CH₂), 1.86 (m, 2H, CH₂), 1.65 (m, 2H, CH₂).

25 MS *m/z* 421 [M⁺+1].

Example 32

1-(2-Oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-6,7-dihydro-2H-pyrano[3,4-*c*]pyrrol-4-one

30 A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (117 mg, 0.54 mmol), 4-oxo-2,4,6,7-tetrahydropyrano[3,4-*c*]pyrrole-1-carbaldehyde (89.3 mg, 0.54 mmol) and piperidine (1 drop) in ethanol (3 mL) was stirred at room temperature for 2 days. The precipitate was collected by vacuum filtration, washed with

ethanol and dried to give 54.1 mg (28%) of the title compound as a yellow solid.

¹HNMR (360 MHz, DMSO-*d*₆) δ 14.0 (br s, 1H, NH), 11.06 (br s, 1H, NH), 7.90 (d, *J* = 2.8 Hz, 1H), 7.44 (s, 1H, H-vinyl),
5 7.16 (t, *J* = 8 Hz, 1H), 6.95 (d, *J* = 8 Hz, 1H), 6.77 (d, *J* = 8 Hz, 1H), 4.34 (t, *J* = 65.7 Hz, 2H, CH₂), 2.29 (t, *J* = 5.7 Hz, 2H, CH₂).

MS *m/z* 364 [*M*⁺+1].

10

Example 33

1-(2-Oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-2,5,6,7-tetrahydropyrrolo[3,4-c]pyridin-4-one

A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (79
15 mg, 0.36 mmol), 4-oxo-4,5,6,7-tetrahydro-2*H*-pyrrolo[3,4-
c]pyridine-1-carbaldehyde (50 mg, 0.3 mmol) and piperidine (1 drop) in ethanol (2 mL) was stirred at room temperature for 4 days. The precipitate was collected by vacuum filtration, washed with ethanol followed by 0.1% of acetic acid in water
20 and dried to give 30 mg (23%) of the title compound.

¹HNMR (360 MHz, DMSO-*d*₆) δ 13.8 (br s, 1H, NH), 11.0 (br s, 1H, NH), 7.66 (d, *J* = 2.9 Hz, 1H), 7.45 (s, 1H, H-vinyl), 7.31 (br s, 1H, NH), 7.15 (t, *J* = 8 Hz, 1H), 6.93 (d, *J* = 8 Hz, 1H),
6.77 (d, *J* = 8 Hz, 1H), 3.44 (m, 2H, CH₂), 3.22 (m, 1H), 3.14
25 (m, 2H), 2.90 (t, 2H, CH₂), 2.76 (t, 2H, CH₂), 1.85 (m, 2H), 1.63 (m, 2H).

MS APCI +ve 363 [*M*⁺+1].

Example 34

30 *5-Methyl-1-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidene-methyl)-2,5,6,7-tetrahydro-pyrrolo[3,4-c]pyridin-4-one*

A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (58.4 mg, 0.27 mmol), 5-methyl-4-oxo-4,5,6,7-tetrahydro-2*H*-

pyrrolo[3,4-c]pyridine-1-carbaldehyde (48 mg, 0.27 mmol) and piperidine (1 drop) in ethanol (1 mL) was stirred at room temperature for 7 days. The precipitate was collected by vacuum filtration, washed with ethanol and dried to give 58 mg (57%) of the title compound as a yellow solid.

¹H NMR (360 MHz, DMSO-*d*₆) δ 13.81 (br s, 1H, NH), 11.03 (br s, 1H, NH), 8.84 (s, 1H, H-vinyl), 7.16 (t, *J* = 7.7 Hz, 1H), 7.12 (d, *J* = 2.2 Hz, 1H), 6.96 (d, *J* = 7.7 Hz, 1H), 6.75 (d, *J* = 7.7 Hz, 1H), 3.54 (t, 2H), 3.3 (m, 1H), 3.04 (m, 4H), 3.01 (s, 3H, CH₃), 2.80 (t, 2H), 1.85 (m, 2H), 1.6 (m, 2H).

MS *m/z* 377 [*M*⁺+1].

Example 35

3-(3,5-Dimethyl-1*H*-pyrrol-2-ylmethylene)-4-piperidin-4-yl-1,3-dihydroindol-2-one

A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol), 3,5-dimethyl-1*H*-pyrrole-2-carbaldehyde (29 mg, 0.23 mmol) and pyrrolidine (0.006 mL) in ethanol (0.5 mL) was refluxed for 2 hours at 90° C. The reaction was cooled and the precipitate which formed was collected by vacuum filtration, washed with ethanol and dried to give the title compound.

¹H NMR (360 MHz, DMSO-*d*₆) δ 13.52 (br s, 1H, NH), 10.82 (br s, 1H, NH), 7.48 (s, 1H, H-vinyl), 7.07 (t, *J* = 7.8 Hz, 1H), 6.91 (d, *J* = 7.8 Hz, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 6.03 (d, *J* = 2.2 Hz, 1H), 3.16 (m, 1H), 3.1 (m, 2H), 2.69 (m, 2H), 2.32 (s, 3H, CH₃), 2.28 (s, 3H, CH₃), 1.83 (m, 2H), 1.60 (m, 2H).

MS +ve APCI *m/z* 322 [*M*⁺+1].

Example 36

3-[2-(2-Oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid

5

A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol), 3-(2-formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid (57 mg, 0.23 mmol) and pyrrolidine (0.3 mL) in ethanol (0.5 mL) was heated to reflux for 2 hours. Acetic acid (0.05 mL) was added to the reaction and heating was continued for another 10 minutes. The reaction was cooled and the precipitate which formed was collected by vacuum filtration, washed with ethanol and dried to give the title compound.

10

MS +ve APCI m/z 420 [M⁺+1].

15

Example 37

3-[5-Methyl-2-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid

20

4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 3-(2-formyl-5-methyl-1H-pyrrol-3-yl)-propionic acid (42 mg, 0.23 mmol) to give the title compound.

25

¹H NMR (360 MHz, DMSO-d₆) δ 10.85 (br s, 1H), 7.58 (s, 1H, H-vinyl), 7.08 (t, J = 7.7 Hz, 1H), 6.90 (d, J = 7.7 Hz, 1H), 6.76 (d, J = 7.7 Hz, 1H), 6.06 (d, J = 1.8 Hz, 1H), 3.19 (m, 2H), 2.86-3.0 (m, 5H), 2.42 (m, 2H), 2.33 (s, 3H, CH₃), 1.91 (m, 2H), 1.73 (m, 2H).

MS +ve APCI m/z 380 [M⁺+1].

30

Example 38

3-[5-Ethyl-2-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid

4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 3-(5-ethyl-2-formyl-1H-pyrrol-3-yl)-

35

propionic acid (0.23 mmol) to give the title compound.

MS +ve APCI m/z 394 [$M^+ + 1$].

Example 39

5 4-(2-Carboxyethyl)-2-methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

10 4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 4-(2-carboxyethyl)-5-formyl-2-methyl-1H-pyrrole-3-carboxylic acid ethyl ester (56 mg, 0.23 mmol) to give the title compound.

MS +ve APCI m/z 452 [$M^+ + 1$].

Example 40

15 3-[2,4-Dimethyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid

20 4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 3-(5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)-propionic acid (43 mg, 0.23 mmol) to give the title compound.

25 ^1H NMR (360 MHz, DMSO- d_6) δ 13.58 (s, 1H, NH), 10.80 (s, 1H, NH), 7.47 (s, 1H, H-vinyl), 7.06 (t, $J = 7.7$ Hz, 1H), 6.89 (d, $J = 7.7$ Hz, 1H), 6.75 (d, $J = 7.7$ Hz, 1H), 3.22 (m, 1H), 3.15 (m, 2H), 2.77 (m, 2H), 2.64 (m, 2H), 2.3 (m, 2H), 2.29 (s, 3H, CH₃), 2.23 (s, 3H, CH₃), 1.87 (m, 2H), 1.68 (m, 2H).

MS +ve APCI m/z 394 [$M^+ + 1$].

Example 41

30 [2,4-Dimethyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-acetic acid

4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with (5-formyl-2,4-dimethyl-1H-pyrrol-3-yl)-

acetic acid (40 mg, 0.23 mmol) to give the title compound.

MS +ve APCI m/z 380 [$M^+ + 1$].

Example 42

5 3-(1*H*-Indol-2-ylmethylene)-4-piperidin-4-yl-1,3-dihydroindol-
2-one

A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 1*H*-indole-2-carbaldehyde (32 mg, 0.23 mmol) to give the title compound.

^1H NMR (360 MHz, DMSO- d_6) δ 13.12 (s, 1H, NH), 7.70 (s, 1H, H-vinyl), 7.66 (d, J = 8.1 Hz, 1H), 7.60 (d, J = 8.1 Hz, 1H), 7.28 (t, 1H), 7.20 (m, 2H), 7.08 (t, J = 7.7 Hz, 1H), 6.95 (d, J = 7.7 Hz, 1H), 6.80 (d, J = 7.7 Hz, 1H), 3.25 (m, 15 3H), 3.0 (m, 2H), 1.93 (m, 2H), 1.76 (m, 2H).

MS +ve APCI m/z 344 [$M^+ + 1$].

Example 43

20 4-Piperidin-4-yl-3-(4,5,6,7-tetrahydro-1*H*-indol-2-
ylmethylene)-1,3-dihydroindol-2-one

4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 4,5,6,7-tetrahydro-1*H*-indole-2-carbaldehyde (33 mg, 0.23 mmol) to give the title compound.

25 ^1H NMR (400 MHz, DMSO- d_6) δ 13.25 (s, 1H, NH), 10.84 (s, 1H, NH), 7.43 (s, 1H, H-vinyl), 7.11 (t, J = 7.7 Hz, 1H), 6.87 (d, J = 7.7 Hz, 1H), 6.76 (d, J = 7.7 Hz, 1H), 6.58 (s, 1H), 3.4 (m, 3H), 2.92 (m, 2H), 2.71 (m, 2H), 1.7-1.9 (m, 10H).

MS -ve APCI 346 [$M^+ - 1$].

30

Example 44

3-[5-(2-Morpholin-4-yl-ethoxy)-1H-indol-2-ylmethylene]-4-piperidin-4-yl-1,3-dihydroindol-2-one

5 4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with (5-(2-morpholin-4-yl-ethoxy)-1H-indole-2-carbaldehyde (60 mg, 0.23 mmol) to give the title compound.

¹H NMR (360 MHz, DMSO-*d*₆) δ 13.04 (s, 1H, NH), 11.1 (br s, 1H, NH), 7.66 (s, 1H, H-vinyl), 7.51 (d, *J* = 8.7 Hz, 1H), 7.2 (t, *J* = 7.8 Hz, 1H), 7.12 (br s, 1H), 7.06 (br s, 1H), 6.94 (m, 2H), 6.79 (d, *J* = 7.8 Hz, 1H), 4.10 (t, 2H, CH₂), 3.59 (t, 4H, 2xCH₂), 3.23 (m, 3H), 2.99 (m, 2H), 2.71 (t, 2H, CH₂), 2.5 (m, 4H, 2xCH₂), 1.93 (m, 2H), 1.76 (m, 2H).

MS +ve APCI *m/z* 473 [M⁺+1].

Example 45

4-Methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid

20 4-Piperidin-4-yl-1,3-dihydro-indol-2-one (45 mg, 0.2 mmol) was condensed with 5-formyl-4-methyl-1H-pyrrole-2-carboxylic acid (34 mg, 0.23 mmol) to give the title compound.

MS +ve APCI *m/z* 352 [M⁺+1].

Example 46

5-Methyl-2-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid

30 4-Piperidin-4-yl-1,3-dihydroindol-2-onem (45 mg, 0.2 mmol) was condensed with 2-formyl-5-methyl-1H-pyrrole-3-carboxylic acid (34 mg, 0.23 mmol) to give the title compound.

MS +APCI *m/z* 352 [M⁺+1].

Example 47

3-[3-(3-Morpholin-4-yl-propyl)-4,5,6,7-tetrahydro-1H-indol-2-ylmethylene]-4-piperidin-4-yl-1,3-dihydroindol-2-one

5 4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 3-(3-morpholin-4-yl-propyl)-4,5,6,7-tetrahydro-1H-indole-2-carbaldehyde (61 mg, 0.23 mmol) to give the title compound.

¹H NMR (360 MHz, DMSO-*d*₆) δ 13.51 (s, 1H, NH), 10.81 (s, 1H, NH), 7.49 (s, 1H, H-vinyl), 7.08 (t, *J* = 7.5 Hz, 1H), 6.90 (d, *J* = 7.5 Hz, 1H), 6.76 (d, *J* = 7.5 Hz, 1H), 3.51 (m, 4H), 3.25 (m, 3H), 2.81 (t, 2H), 2.68 (m, 2H), 2.62 (m, 2H), 2.44 (m, 2H), 2.28 (m, 6H), 1.64-1.92 (m, 10H).

MS +ve APCI *m/z* 475 [*M*⁺+1].

Example 48

*2,4-Dimethyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid
(2-diethylamino-ethyl)-amide*

20 4-Piperidin-4-yl-1,3-dihydroindol-2-one was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)-amide to give the title compound.

¹H NMR (360 MHz, DMSO-*d*₆) δ 13.79 (s, 1H, NH), 11.0 (br s, 1H, NH), 7.51 (s, 1H, H-vinyl), 7.44 (t, *J* = 5.6 Hz, 1H, CONHCH₂), 7.11 (t, *J* = 7.7 Hz, 1H), 6.93 (d, *J* = 7.7 Hz, 1H), 6.77 (d, *J* = 7.7 Hz, 1H), 3.3 (m, 4H), 2.72 (m, 2H), 2.52 (q, *J* = 7.2 Hz, 4H, N(CH₂CH₃)₂), 2.43 (s, 3H, CH₃), 2.37 (s, 3H, CH₃), 1.85 (m, 2H), 1.64 (m, 2H), 0.97 (t, *J* = 7.2 Hz, 6H, N(CH₂CH₃)₂).

MS +ve APCI *m/z* 464 [*M*⁺+1].

Example 49

3-[3-Methyl-5-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-2-ylmethylenel]-4-piperidin-4-yl-1,3-dihydroindol-2-one

5 4-Piperidin-4-yl-1,3-dihydro-indol-2-one (45 mg, 0.2 mmol) was condensed with 3-methyl-5-(4-methylpiperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (52 mg, 0.23 mmol) to give the title compound.

10 ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.80 (s, 1H, NH), 7.51 (s, 1H, H-vinyl), 7.16 (t, 1H), 6.95 (d, 1H), 6.77 (d, 1H), 6.60 (d, 1H), 3.67 (m, 4H), 3.1-3.25 (m, 3H), 2.73 (m, 2H), 2.36 (m, 4H), 2.21 (s, 3H, CH₃), 2.19 (s, 3H, CH₃), 1.85 (m, 2H), 1.61 (m, 2H).

MS +ve APCI *m/z* 434 [M⁺+1].

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Example 50

3-(2-Oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid ethyl ester

20 4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 3-formyl-4,5,6,7-tetrahydro-2H-isoindole-1-carboxylic acid ethyl ester (48 mg, 0.23 mmol) to give the title compound.

25 ¹H NMR (360 MHz, DMSO-*d*₆) δ 13.89 (s, 1H, NH), 11.1 (br s, 1H, NH), 7.40 (s, 1H, H-vinyl), 7.19 (t, *J* = 7.7 Hz, 1H), 6.94 (d, *J* = 7.7 Hz, 1H), 6.79 (d, *J* = 7.7 Hz, 1H), 4.28 (q, *J* = 7.1 Hz, 2H, OCH₂CH₃), 3.25 (m, 3H), 2.87 (m, 2H), 2.72 (m, 4H), 1.90 (m, 2H), 1.73 (m, 6H), 1.31 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃).

MS +ve APCI *m/z* 420 [M⁺+1].

30

Example 51

2-Methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-4-phenyl-1H-pyrrole-3-carboxylic acid pyridin-4-ylamide

5

4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 5-formyl-2-methyl-4-phenyl-1H-pyrrole-3-carboxylic acid pyridin-4-ylamide (67 mg, 0.23 mmol) to give the title compound.

10

¹H NMR (360 MHz, DMSO-*d*₆) δ 14.15 (s, 1H, NH), 11.05 (br s, 1H, NH), 9.78 (s, 1H), 8.36 (d, *J* = 6.2 Hz, 2H), 7.44 (m, 9H), 7.12 (t, *J* = 7.8 Hz, 1H), 6.85 (d, *J* = 7.8 Hz, 1H), 6.77 (d, *J* = 7.8 Hz, 1H), 2.71 (m, 3H), 2.54 (s, 3H, CH₃), 1.76 (m, 2H), 1.58 (m, 2H).

15

MS +ve APCI *m/z* 504 [M⁺+1].

Example 52

3-(5-Methylthiophen-2-ylmethylene)-4-piperidin-4-yl-1,3-dihydro-indol-2-one

20

4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 5-methylthiophene-2-carbaldehyde (28 mg, 0.23 mmol) to give the title compound.

MS +ve APCI *m/z* 325 [M⁺+1].

25

Example 53

3-(4-Morpholin-4-yl-benzylidene)-4-piperidin-4-yl-1,3-dihydro-indol-2-one

30

4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 4-morpholin-4-yl-benzaldehyde (43 mg, 0.23 mmol) to give the title compound.

¹H NMR (360 MHz, DMSO-*d*₆) δ 10.48 (s, 1H, NH), 8.18 (d, *J* = 8.9 Hz, 2H), 7.52 (s, 1H, H-vinyl), 7.12 (t, *J* = 8.0 Hz,

1H), 6.98 (d, $J = 8.9$ Hz, 2H), 6.87 (d, $J = 8.0$ Hz, 1H), 6.67 (d, $J = 8.0$ Hz, 1H), 3.73 (m, 4H), 3.35 (m, 4H), 3.2 (m, 3H), 2.79 (m, 2H), 1.86 (m, 2H), 1.72 (m, 2H).

MS +ve APCI m/z 390 [$M^+ + 1$].

5

Example 54

4-(2-Carboxyethyl)-3-methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-2-carboxylic acid ethyl ester

10

4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 4-(2-carboxyethyl)-5-formyl-3-methyl-1H-pyrrole-2-carboxylic acid ethyl ester (55 mg, 0.23 mmol) to give the title compound.

15

MS +ve APCI m/z 452 [$M^+ + 1$].

Example 55

4-(2-Hydroxyethyl)-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid

20

A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one was condensed with 5-formyl-4-(2-hydroxyethyl)-1H-pyrrole-3-carboxylic acid to give the title compound.

MS +ve APCI m/z 382 [$M^+ + 1$].

25

Example 56

4-(4-Methoxyphenyl)-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

30

4-Piperidin-4-yl-1,3-dihydroindol-2-one (45 mg, 0.2 mmol) was condensed with 5-formyl-4-(4-methoxyphenyl)-1H-pyrrole-3-carboxylic acid ethyl ester (60 mg, 0.23 mmol) to give the title compound.

¹HNMR (360 MHz, DMSO-d₆) δ 14.13 (s, 1H, NH), 11.03 (br s, 1H, NH), 7.35 (s, 1H, H-vinyl), 7.25 (d, J = 8.7 Hz, 2H), 7.10 (t, J = 7.8 Hz, 1H), 7.0 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 7.8 Hz, 1H), 6.74 (d, J = 7.8 Hz, 1H), 4.02 (q, J = 7.0 Hz, 3H, OCH₂CH₃), 3.81 (s, 3H, OCH₃), 2.75 (m, 3H), 1.83 (m, 2H), 1.52 (m, 2H), 1.37 (m, 2H), 1.04 (t, J = 7.0 Hz, 3H, OCH₂CH₃).

Example 57

10 4-Methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidene-methyl)-1H-pyrrole-3-carboxylic acid benzylamide

A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (54 mg, 0.25 mmol), 5-formyl-4-methyl-1H-pyrrole-3-carboxylic acid benzylamide (63.5 mg, 0.2625 mmol) and piperidine (2 drops) in 15 ethanol (0.5 mL) was stirred at room temperature for 7 days. The precipitate which formed was collected by vacuum filtration, washed with ethanol and dried to give the title compound.

20 ¹HNMR (360 MHz, DMSO-d₆) δ 11.08 (br s, 1H, NH), 8.54 (t, 1H), 7.86 (d, 1H), 7.50 (s, 1H), 7.32 (m, 5H), 7.18 (t, 1H), 6.91 (d, 1H), 6.82 (d, 1H), 4.43 (d, 2H), 3.44 (m, 3H), 3.08 (t, 2H), 2.55 (s, 3H, CH₃), 2.06 (m, 2H), 1.92 (m, 2H).

MS -ve APCI m/z 439 [M⁺-1].

25

Example 58

4-Methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid
(pyridin-4-ylmethyl)-amide

30 A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (54 mg, 0.25 mmol), 5-formyl-4-methyl-1H-pyrrole-3-carboxylic acid (pyridin-4-ylmethyl)-amide (64 mg, 0.2625 mmol) and piperidine (2 drops) in ethanol (0.5 mL) was stirred at room temperature for 7 days. The precipitate which formed was collected by 35 vacuum filtration, washed with ethanol and dried to give the

title compound.

¹HNMR (360 MHz, DMSO-d₆) δ 11.08 (br s, 1H, NH), 8.64 (t, 1H), 8.51 (d, 2H), 7.88 (m, 1H), 7.51 (s, 1H), 7.31 (d, 2H), 7.19 (t, 1H), 6.92 (d, 1H), 6.82 (d, 1H), 4.44 (m, 2H), 3.55 (m, 3H), 3.07 (m, 2H), 2.54 (s, 3H, CH₃), 2.06 (m, 2H), 1.90 (m, 2H).

MS -ve APCI m/z 440 [M⁺-1].

Example 59

10 *4-Methyl-5-(2-oxo-4-piperidin-4-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid [3-(2-oxo-pyrrolidin-1-yl)-propyl]-amide*

A mixture of 4-piperidin-4-yl-1,3-dihydroindol-2-one (35.6 mg, 0.165 mmol), 5-formyl-4-methyl-1H-pyrrole-3-carboxylic acid [3-(2-oxo-pyrrolidin-1-yl)propyl]-amide (48 mg, 0.173 mmol) and piperidine (2 drops) in ethanol (0.5 mL) was stirred at room temperature for 7 days. The reaction was concentrated and ether was added to the residue. The precipitate which formed was collected by vacuum filtration, washed with ethanol and ether and dried to give the title compound.

¹HNMR (360 MHz, DMSO-d₆) δ 11.07 (s, 1H, NH), 8.05 (br s, 1H), 7.94 (t, 1H, NH), 7.76 (d, 1H), 7.49 (s, 1H), 7.18 (t, 1H), 6.91 (d, 1H), 6.82 (d, 1H), 3.47 (m, 3H), 3.35 (m, 2H), 3.15 (m, 6H), 2.53 (s, 3H, CH₃), 2.22 (m, 2H), 2.08 (m, 2H), 1.93 (m, 2H), 1.67 (m, 2H).

MS -ve APCI m/z 474 [M⁺-1].

Example 60

30 *2-Methyl-4-[3-(4-methylpiperazin-1-yl)-propyl]-5-(2-oxo-4-pyridin-2-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester*

35 A mixture of 4-pyridin-2-yl-1,3-dihydroindol-2-one (31.5

mg, 0.15 mmol), 5-formyl-2-methyl-4-[3-(4-methylpiperazin-1-yl)-propyl]-1H-pyrrole-3-carboxylic acid ethyl ester (48.2 mg, 0.15 mmol) and piperidine (0.1 mL) in ethanol (1 mL) was heated in a sealed tube at 70° C for 6 hours. The reaction was concentrated and the residue was re-crystallized from ethyl acetate and hexane to give the title compound.

¹HNMR (360 MHz, DMSO-*d*₆) δ 13.92 (s, 1H, NH), 11.2 (br s, 1H, NH), 8.74 (d, *J* = 4.4 Hz, 1H), 7.99 (dt, *J* = 1.8 & 7.6 Hz, 1H), 7.61 (d, *J* = 7.5 Hz, 1H), 7.54 (m, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 6.98 (m, 2H), 6.90 (d, *J* = 7.2 Hz, 1H), 4.17 (q, *J* = 7.0 Hz, 2H, OCH₂CH₃), 2.50 (s, 3H, CH₃), 2.27 (m, 10 H), 2.11 (s, 3H, CH₃), 1.99 (br t, 2H), 1.3 (m, 2H), 1.26 (t, *J* = 7.0 Hz, 3H, OCH₂CH₃).

MS -ve APCI *m/z* 512 [M⁺-1].

Example 61

3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-pyridin-2-yl-1,3-dihydroindol-2-one

A mixture of 4-pyridin-2-yl-1,3-dihydroindol-2-one (31.5 mg, 0.15 mmol), 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (37.4 mg, 0.15 mmol) and piperidine (0.1 mL) in ethanol (1 mL) was heated in a sealed tube at 70° C for 6 hours. The reaction was concentrated and the residue was re-crystallized from ethyl acetate and hexane to give the title compound.

¹HNMR (360 MHz, DMSO-*d*₆) δ 13.53 (s, 1H, NH), 11.06 (br s, 1H, NH), 8.74 (d, *J* = 4.0 Hz, 1H), 7.99 (dt, *J* = 1.7 & 7.7 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.50 (m, 1H), 7.22 (t, *J* = 7.7 Hz, 1H), 6.97 (d, *J* = 7.7 Hz, 1H), 6.90 (d, *J* = 7.7 Hz, 1H), 6.85 (s, 1H), 3.32 (s, 3H, CH₃), 2.24 (s, 8H), 2.16 (s, 3H, CH₃), 1.64 (s, 3H, CH₃).

MS -ve APCI *m/z* 440 [M⁺-1].

Example 62

3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-pyrimidin-5-yl-1,3-dihydroindol-2-one

5 4-Pyrimidin-5-yl-1,3-dihydroindol-2-one (53 mg, 0.25 mmol) was condensed with 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (69 mg, 0.275 mmol) to give the title compound.

MS +ve APCI m/z 443 [$M^+ + 1$].

Example 63

3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-thiazol-2-yl-1,3-dihydroindol-2-one

15 4-Thiazol-2-yl-1,3dihydro-indol-2-one (54 mg, 0.25 mmol) was condensed with 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (69 mg, 0.275 mmol) to give the title compound.

MS +ve APCI m/z 448 [$M^+ + 1$].

Example 64

2-Methyl-4-[3-(4-methylpiperazin-1-yl)propyl]-5-(2-oxo-4-pyrimidin-5-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

25 4-Pyrimidin-5-yl-1,3-dihydroindol-2-one (53 mg, 0.25 mmol) was condensed with 5-formyl-2-methyl-4-[3-(4-methylpiperazin-1-yl)-propyl]-1H-pyrrole-3-carboxylic acid ethyl ester (88 mg, 0.275 mmol) to give the title compound.

30 MS +ve APCI m/z 515 [$M^+ + 1$].

Example 65

2-Methyl-4-[3-(4-methylpiperazin-1-yl)propyl]-5-(2-oxo-4-thiazol-2-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

5

4-Thiazol-2-yl-1,3-dihydroindol-2-one (54 mg, 0.25 mmol) was condensed with 5-formyl-2-methyl-4-[3-(4-methylpiperazin-1-yl)propyl]-1H-pyrrole-3-carboxylic acid ethyl ester (88 mg, 0.275 mmol) to give the title compound.

10

¹HNMR (360 MHz, DMSO-*d*₆) δ 13.96 (br s, 1H, NH), 11.22 (br s, 1H, NH), 8.15 (s, 1H, H-vinyl), 8.09 (d, *J* = 3.3 Hz, 1H), 8.02 (d, *J* = 3.3 Hz, 1H), 7.27 (t, *J* = 7.7 Hz, 1H), 7.17 (d, *J* = 7.7 Hz, 1H), 7.06 (d, *J* = 7.7 Hz, 1H), 4.20 (q, 2H), 3.43 (m, 2H), 2.62 (t, 2H), 2.53 (s, 3H, CH₃), 2.24 (m, 8H), 2.09 (s, 3H, CH₃), 1.48 (m, 2H), 1.05 (t, 3H).

15

Example 66

5-[4-(6-Aminopyridin-3-yl)-2-oxo-1,2-dihydroindol-3-ylidene-methyl]-2-methyl-4-[3-(4-methylpiperazin-1-yl)propyl]-1H-pyrrole-3-carboxylic acid ethyl ester

20

4-(6-Aminopyridin-3-yl)-1,3-dihydroindol-2-one (56 mg, 0.25 mmol) was condensed with 5-formyl-2-methyl-4-[3-(4-methyl-piperazin-1-yl)propyl]-1H-pyrrole-3-carboxylic acid ethyl ester (88 mg, 0.275 mmol) to give the title compound.

25

¹HNMR (360 MHz, DMSO-*d*₆) δ 13.84 (br s, 1H, NH), 11.06 (br s, 1H, NH), 7.95 (d, 1H), 7.44 (dd, 1H), 7.24 (s, 1H), 7.17 (t, 1H), 6.88 (d, 1H), 6.74 (d, 1H), 6.58 (d, 1H), 6.14 (d, 1H), 4.18 (q, 2H), 3.27 (s, 3H, CH₃), 2.40 (m, 2H), 2.25 (m, 8H), 2.10 (s, 3H, CH₃), 2.08 (m, 2H), 1.34 (m, 2H), 1.27 (t, 3H).

30

MS -ve APCI *m/z* 527 [M⁺-1].

Example 67

4-(6-Aminopyridin-3-yl)-3-[3,5-dimethyl-4-(4-methylpiperazin-1-ylcarbonyl)-1H-pyrrol-2-ylmethylene]-1,3-dihydroindol-2-one

5 4-(6-Aminopyridin-3-yl)-1,3-dihydroindol-2-one (56 mg, 0.25 mmol) was condensed with 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (69 mg, 0.275 mmol) to give the title compound.

MS -ve APCI m/z 455 [M⁺-1].

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Example 68

2-Methyl-4-[3-(4-methylpiperazin-1-yl)propyl]-5-(2-oxo-4-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrole-3-carboxylic acid ethyl ester

15

A mixture of 4-pyridin-3-yl-1,3-dihydroindol-2-one (42 mg, 0.2 mmol), 5-formyl-2-methyl-4-[3-(4-methylpiperazin-1-yl)-propyl]-1H-pyrrole-3-carboxylic acid ethyl ester (64 mg, 0.2 mmol) and piperidine (0.1 mL) in ethanol was heated in a sealed tube at 70° C for 5 hours. The reaction was concentrated and the residue was purified by column chromatography to give the title compound.

20

¹H NMR (360 MHz, DMSO-d₆) δ 13.84 (s, 1H, NH), 11.20 (s, 1H, NH), 8.75 (dd, J = 1.6 & 4.7 Hz, 1H), 8.68 (d, J = 1.6 Hz, 1H), 7.93 (dt, 1H), 7.60 (dd, 1H), 7.26 (t, J = 7.6 Hz, 1H), 6.99 (d, J = 7.6 Hz, 1H), 6.83 (d, J = 7.6 Hz, 1H), 6.80 (s, 1H, H-vinyl), 4.17 (q, J = 7.1 Hz, 2H, OCH₂CH₃), 2.4-2.75 (m, 11H), 2.38 (s, 3H, CH₃), 2.17 (m, 4H), 1.30 (m, 2H), 1.26 (t, J = 7.1 Hz, 3H, OCH₂CH₃).

25

30 MS -ve APCI m/z 512 [M⁺-1].

Example 69

3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-4-pyridin-3-yl-1,3-dihydroindol-2-one

5 A mixture of 4-pyridin-3-yl-1,3-dihydroindol-2-one (42 mg, 0.2 mmol), 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (50 mg, 0.2 mmol) and piperidine (0.1 mL) in ethanol was heated in a sealed tube at 70° C for 5 hours. The reaction was concentrated and the residue was
10 crystallized from ethyl acetate/ hexane to give the title compound.

¹H NMR (360 MHz, DMSO-*d*₆) δ 13.44 (s, 1H, NH), 11.08 (s, 1H, NH), 8.75 (dd, 1H), 8.64 (d, 1H), 7.89 (br d, 1H), 7.58 (dd, 1H), 7.23 (t, *J* = 7.7 Hz, 1H), 6.98 (d, *J* = 7.7 Hz, 1H),
15 6.83 (d, *J* = 7.7 Hz, 1H), 6.66 (s, 1H, H-vinyl), 3.4 (m, 4H), 2.24 (br s, 7H), 2.16 (s, 3H, CH₃), 1.55 (s, 3H, CH₃).

MS -ve APCI *m/z* 440 [M⁺-1].

Example 70

20 5-(3-{4-Ethoxycarbonyl-5-methyl-3-[3-(4-methylpiperazin-1-yl)-propyl]-1H-pyrrol-2-ylmethylene}-2-oxo-2,3-dihydro-1H-indol-4-yl)-nicotinic acid

A mixture of 5-(2-oxo-2,3-dihydro-1H-indol-4-yl)-
25 nicotinic acid (51 mg, 0.2 mmol), 5-formyl-2-methyl-4-[3-(4-methyl-piperazin-1-yl)-propyl]-1H-pyrrole-3-carboxylic acid ethyl ester (64 mg, 0.2 mmol) and piperidine (0.2 mL) in ethanol (1mL) was heated in a sealed tube at 60° C for 5 hours. The reaction was acidified with 1N HCl and the resulted
30 precipitate was collected by vacuum filtration, washed with water and dried to give the title compound.

¹H NMR (360 MHz, DMSO-*d*₆) δ 13.88 (s, 1H, NH), 11.22 (s, 1H, NH), 9.18 (d, *J* = 2.1 Hz, 1H), 8.71 (d, *J* = 2.1 Hz, 1H), 8.24 (m, 1H), 7.26 (t, *J* = 7.6 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 1H),
35 6.84 (d, *J* = 7.6 Hz, 1H), 6.77 (s, 1H, H-vinyl), 4.14 (q,

$J = 7.4$ Hz, 2H, OCH_2CH_3), 2.72 (m, 8H), 2.5 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.2-2.4 (m, 4H), 1.25 (m, 2H), 1.23 (t, $J = 7.4$ Hz, 3H, OCH_2CH_3).

MS +ve APLCI m/z 558 $[\text{M}^+ + 1]$.

5

Example 71

5-(3-[3,5-Dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indol-4-yl)-nicotinic acid

10

A mixture of 5-(2-oxo-2,3-dihydro-1H-indol-4-yl)-nicotinic acid (381 mg, 1.5 mmol), 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (374 mg, 1.5 mmol) and piperidine (1 mL) in ethanol (5 mL) was heated in a sealed cube at 60°C for 5 hours. The reaction was acidified with 1N HCl and the resulted precipitate was collected by vacuum filtration, washed with water and dried to give the title compound.

15

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 13.49 (s, 1H, NH), 11.61 (s, 1H, NH), 9.18 (s, 1H), 8.88 (s, 1H), 8.30 (s, 1H), 7.26 (m, 1H), 7.01 (d, $J = 7.4$ Hz, 1H), 6.89 (d, $J = 7.4$ Hz, 1H), 6.66 (s, 1H, H-vinyl), 3.58 (br s, 4H), 2.91 (br s, 4H), 2.61 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 1.55 (s, 3H, CH_3).

20

MS -ve APCI m/z 484 $[\text{M}^+ - 1]$.

25

Example 72

5-(3-[4-(2-Diethylaminoethylcarbonyl)-3,5-dimethyl-1H-pyrrol-2-ylmethylene]-2-oxo-2,3-dihydro-1H-indol-4-yl)-nicotinic acid

A mixture of 5-(2-oxo-2,3-dihydro-1H-indol-4-yl)-nicotinic acid (381 mg, 1.5 mmol), 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)-amide (398 mg, 1.5 mmol) and piperidine (1 mL) in ethanol (5 mL) was heated in a sealed tube at 60°C for 10 hours. The reaction was concentrated, the residue was dissolved in a mixture of water and methanol and then acidified with 1N HCl. The resulted

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35

precipitate was collected by vacuum filtration, washed with methanol and dried to give the title compound.

¹H NMR (360 MHz, DMSO-*d*₆) δ 13.54 (s, 1H, NH), 11.15 (s, 1H, NH), 9.19 (d, *J* = 2 Hz, 1H), 8.88 (d, *J* = 2 Hz, 1H), 8.3 (m, 1H), 7.72 (br t, *J* = 5.8 Hz, 1H), 7.26 (t, *J* = 7.7 Hz, 1H), 7.01 (d, *J* = 7.7 Hz, 1H), 6.89 (d, *J* = 7.7 Hz, 1H), 6.66 (s, 1H, H-vinyl), 3.52 (m, 2H), 3.18 (m, 6H), 2.43 (s, 3H, CH₃), 1.69 (s, 3H, CH₃), 1.21 (t, *J* = 7.4 Hz, 6H, N(CH₂CH₃)₂).

MS +ve APCI *m/z* 502 [M⁺+1].

Example 73

5-[4-(2-Aminopyrimidin-5-yl)-2-oxo-1,2-dihydroindol-3-ylidene-methyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid
(2-diethylaminoethyl)amide

4-(2-Aminopyrimidin-5-yl)-1,3-dihydroindol-2-one (55 mg) was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (65 mg) to give the title compound.

¹H NMR (360 MHz, DMSO-*d*₆) δ 13.46 (s, 1H, NH), 11.02 (br s, 1H, NH), 8.27 (s, 2H), 7.38 (t, 1H, NH), 7.17 (t, *J* = 7.8 Hz, 1H), 7.02 (s, 1H, H-vinyl), 6.91 (d, *J* = 7.8 Hz, 1H), 6.86 (s, 2H, NH₂), 6.79 (d, *J* = 7.8 Hz, 1H), 3.22 (m, 2H), 2.45-2.52 (m, 6H), 2.39 (s, 3H, CH₃), 1.90 (s, 3H, CH₃), 0.95 (t, *J* = 7.0 Hz, 6H, N(CH₂CH₃)₂).

MS -ve APCI *m/z* 472 [M⁺-1].

Example 74

4-(2-Aminopyrimidin-5-yl)-3-[3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrol-2-ylmethylene]-1,3-dihydroindol-2-one

4-(2-Aminopyrimidin-5-yl)-1,3-dihydroindol-2-one (89 mg) was condensed with 3,5-dimethyl-4-(4-methylpiperazine-1-carbonyl)-1H-pyrrole-2-carbaldehyde (98 mg) to give the title

compound.

¹H NMR (360 MHz, DMSO-*d*₆) δ 13.42 (s, 1H, NH), 11.01 (br s, 1H, NH), 8.28 (s, 2H), 7.17 (t, *J* = 7.7 Hz, 1H), 6.98 (s, 1H, H-vinyl), 6.91 (d, *J* = 7.7 Hz, 1H), 6.86 (s, 2H, NH₂), 6.79 (d, *J* = 7.7 Hz, 1H), 3.3-3.4 (br s, 4H), 2.24 (br s, 4H), 2.16 (s, 3H, CH₃), 1.76 (s, 3H, CH₃).

MS -ve APCI *m/z* 456 [*M*⁺-1].

Example 75

10 2,4-Dimethyl-5-(2-oxo-4-pyridin-3-yl-1,2-dihydroindol-3-ylidenemethyl)-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide

15 A mixture of 4-pyridin-3-yl-1,3-dihydroindol-2-one (42 mg, 0.2 mmol), 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (53 mg, 0.2 mmol) and piperidine (0.1 mL) in ethanol (1 mL) was heated in a sealed tube at 80°C for 2 hours, heating was stopped and the tube was shaken at room temperature for 2 days. A small amount of water
20 was added to the reaction and the solid which formed was collected by vacuum filtration, washed with water and dried to give the title compound.

¹H NMR (400 MHz, DMSO-*d*₆) δ 13.47 (s, 1H, NH), 11.10 (s, 1H, NH), 8.71 (dd, *J* = 1.5 & 4.7 Hz, 1H), 8.64 (d, *J* = 1.5 Hz, 1H), 7.89 (m, 1H), 7.58 (m, 1H), 7.36 (t, *J* = 5.5 Hz, 1H, NH), 7.23 (t, *J* = 7.6 Hz, 1H, NH), 6.98 (d, *J* = 7.6 Hz, 1H), 6.83 (d, *J* = 7.6 Hz, 1H), 6.68 (s, 1H, H-vinyl), 3.21 (m, 2H), 2.5 (m, 6H), 2.39 (s, 3H, CH₃), 1.68 (s, 3H, CH₃), 0.95 (t, *J* = 7.2 Hz, 6H, N(CH₂CH₃)₂).

30 MS -ve APCI *m/z* 456 [*M*⁺-1].

BIOLOGICAL EVALUATION

It will be appreciated that, in any given series of compounds, a range of biological activities will be observed. In
35 its presently preferred embodiments, this invention relates to

novel 4-heteroaryl-3-heteroarylidenyl-2-indolinones
demonstrating the ability to modulate RTK, CTK, and STK activity.

The following assays are employed to explore the activity of the
compounds of this invention and to select those demonstrating the
desired level of activity against various target species.

A. Assay Procedures.

The following assays may be used to determine the level
of activity and effect of the different compounds of the
present invention on one or more PKs. Similar assays can be
designed along the same lines for any PK using techniques
well known in the art.

Several of the assays described herein are performed in
an ELISA (Enzyme-Linked Immunosorbent Sandwich Assay) format
(Voller, et al., 1980, "Enzyme-Linked Immunosorbent Assay,"
Manual of Clinical Immunology, 2d ed., Rose and Friedman,
Am. Soc. Of Microbiology, Washington, D.C., pp. 359-371).
The general procedure is as follows: a compound is
introduced to cells expressing the test kinase, either
naturally or recombinantly, for a selected period of time
after which, if the test kinase is a receptor, a ligand
known to activate the receptor is added. The cells are
lysed and the lysate is transferred to the wells of an ELISA
plate previously coated with a specific antibody recognizing
the substrate of the enzymatic phosphorylation reaction.
Non-substrate components of the cell lysate are washed away
and the amount of phosphorylation on the substrate is
detected with an antibody specifically recognizing
phosphotyrosine compared with control cells that were not
contacted with a test compound.

The presently preferred protocols for conducting the
ELISA experiments for specific PKs is provided below.
However, adaptation of these protocols for determining the
activity of compounds against other RTKs, as well as for
CTKs and STKs, is well within the scope of knowledge of
those skilled in the art. Other assays described herein

measure the amount of DNA made in response to activation of a test kinase, which is a general measure of a proliferative response. The general procedure for this assay is as follows: a compound is introduced to cells expressing the

5 test kinase, either naturally or recombinantly, for a selected period of time after which, if the test kinase is a receptor, a ligand known to activate the receptor is added.

After incubation at least overnight, a DNA labeling reagent such as 5-bromodeoxyuridine (BrdU) or H³-thymidine is added.

10 The amount of labeled DNA is detected with either an anti-BrdU antibody or by measuring radioactivity and is compared to control cells not contacted with a test compound.

GST-FLK-1 BIOASSAY

This assay analyzes the tyrosine kinase activity of GST-Flk1 on poly(glu-tyr) peptides.

Materials and Reagents:

1. Corning 96-well ELISA plates (Corning Catalog No. 25805-96).
2. poly(glu-tyr) 4:1, lyophilizate (Sigma Catalog No. P0275), 1 mg/ml in sterile PBS.
3. PBS Buffer: for 1 L, mix 0.2 g KH₂PO₄, 1.15 g Na₂HPO₄, 0.2 g KCl and 8 g NaCl in approx. 900ml dH₂O. When all reagents have dissolved, adjust the pH to 7.2 with HCl. Bring total volume to 1 L with dH₂O.
4. PBST Buffer: to 1 L of PBS Buffer, add 1.0 ml Tween-20.
5. TBB - Blocking Buffer: for 1 L, mix 1.21 g TRIS, 8.77 g NaCl, 1 ml TWEEN-20 in approximately 900 ml dH₂O. Adjust pH to 7.2 with HCl. Add 10 g BSA, stir to dissolve. Bring total volume to 1 L with dH₂O. Filter to remove particulate matter.
6. 1% BSA in PBS: add 10 g BSA to approx. 990 ml PBS buffer, stir to dissolve. Adjust total volume to 1 L with PBS buffer, filter to remove particulate matter.

7. 50 mM Hepes pH 7.5.
8. GST-Flklcd purified from sf9 recombinant baculovirus transformation (SUGEN, Inc.).
9. 4% DMSO in dH₂O.
- 5 10. 10 mM ATP in dH₂O.
11. 40 mM MnCl₂
12. Kinase Dilution Buffer (KDB): mix 10 ml Hepes (pH 7.5), 1 ml 5M NaCl, 40 µL 100 mM sodium orthovanadate and 0.4 ml of 5% BSA in dH₂O with 88.56 ml dH₂O.
- 10 13. NUNC 96-well V bottom polypropylene plates, Applied Scientific Catalog # AS-72092
14. EDTA: mix 14.12 g ethylenediaminetetraacetic acid (EDTA) with approx. 70 ml dH₂O. Add 10 N NaOH until EDTA dissolves. Adjust pH to 8.0. Adjust total volume to 100 ml with dH₂O.
- 15 15. 1^o and 2^o Antibody Dilution Buffer: mix 10 ml of 5% BSA in PBS buffer with 89.5 ml TBST.
16. Anti-phosphotyrosine rabbit polyclonal antisera(Sugen, Inc.)
- 20 17. Goat anti-rabbit HRP conjugate.
18. ABST solution: To approx. 900 ml dH₂O add 19.21 g citric acid and 35.49 g Na₂HPO₄. Adjust pH to 4.0 with phosphoric acid. Add 2,2'-Azinobis(3-ethyl-benzthiazoline-6-sulfonic acid (ABTS, Sigma, Cat. No. A-1888, hold for approx. ½ hour, filter.
- 25 19. 30% Hydrogen Peroxide.
20. ABST/H₂O₂: add 3 µl of H₂O₂ to 15 ml of ABST solution.
21. 0.2 M HCl.

30 Procedure:

1. Coat Corning 96-well ELISA plates with 2 µg of polyEY in 100 µl PBS/well, hold at room temperature for 2 hours or at 4^o C overnight. Cover plates to prevent evaporation.

2. Remove unbound liquid from wells by inverting plate. Wash once with TBST. Pat the plate on a paper towel to remove excess liquid.
3. Add 100 μ l of 1% BSA in PBS to each well. Incubate, with shaking, for 1 hr. at room temperature.
4. Repeat step 2.
5. Soak wells with 50 mM HEPES (pH7.5, 150 μ l/well).
6. Dilute test compound with dH₂O/4% DMSO to 4 times the desired final assay concentration in 96-well polypropylene plates.
7. Add 25 μ l diluted test compound to each well of ELISA plate. In control wells, place 25 μ l of dH₂O/4% DMSO.
8. Dilute GST-Flk1 0.005 μ g (5 ng)/well in KDB.
9. Add 50 μ l of diluted enzyme to each well.
10. Add 25 μ l 0.5 M EDTA to negative control wells.
11. Add 25 μ l of 40 mM MnCl₂ with 4X ATP (2 μ M) to all wells (100 μ l final volume, 0.5 μ M ATP final concentration in each well).
12. Incubate, with shaking, for 15 minutes at room temperature.
13. Stop reaction by adding 25 μ l of 500 mM EDTA to each well.
14. Wash 3X with TBST and pat plate on paper towel to remove excess liquid.
15. Add 100 μ l per well anti-phosphotyrosine antisera, 1:10,000 dilution in antibody dilution buffer. Incubate, with shaking, for 90 min. at room temperature.
16. Wash as in step 14.
17. Add 100 μ l/well of goat anti-rabbit HRP conjugate (1:6,000 in antibody dilution buffer). Incubate, with shaking, for 90 minutes at room temperature.

18. Wash as in Step 14.
19. Add 100 μ l room temperature ABST/H₂O₂ solution to each well.
20. Incubate, with shaking for 15 to 30 minutes at room temperature.
21. If necessary, stop reaction by adding 100 μ l of 0.2 M HCl to each well.
22. Read results on Dynatech MR7000 ELISA reader with test filter at 410 nM and reference filter at 630 nM.

PYK2 BIOASSAY

This assay is used to measure the in vitro kinase activity of HA epitope-tagged full length pyk2 (FL.pyk2-HA) in an ELISA assay.

Materials and Reagents:

1. Corning 96-well Elisa plates.
2. 12CA5 monoclonal anti-HA antibody (SUGEN, Inc.)
3. PBS (Dulbecco's Phosphate-Buffered Saline (Gibco Catalog # 450-1300EB)
4. TBST Buffer: for 1 L, mix 8.766 g NaCl, 6.057 g TRIS and 1 ml of 0.1% Triton X-100 in approx. 900 ml dH₂O. Adjust pH to 7.2, bring volume to 1 L.
5. Blocking Buffer: for 1 L, mix 100 g 10% BSA, 12.1 g 100 mM TRIS, 58.44 g 1M NaCl and 10 mL of 1% TWEEN-20.
6. FL.pyk2-HA from sf9 cell lysates (SUGEN, Inc.).
7. 4% DMSO in MilliQue H₂O.
8. 10 mM ATP in dH₂O.
9. 1M MnCl₂.
10. 1M MgCl₂.
11. 1M Dithiothreitol (DTT).
12. 10X Kinase buffer phosphorylation: mix 5.0 ml 1M Hepes (pH 7.5), 0.2 ml 1M MnCl₂, 1.0 ml 1 M MgCl₂, 1.0 ml 10% Triton X-100 in 2.8 ml dH₂O. Just prior to use, add 0.1 ml 1M DTT.

13. NUNC 96-well V bottom polypropylene plates.
14. 500 mM EDTA in dH₂O.
15. Antibody dilution buffer: for 100 mL, 1 mL 5% BSA/PBS and 1 mL 10% Tween-20 in 88 mL TBS.
- 5 16. HRP-conjugated anti-Ptyr (PY99, Santa Cruz Biotech Cat. No. SC-7020).
17. ABTS, Moss, Cat. No. ABST-2000.
18. 10% SDS.

Procedure:

- 10 1. Coat Corning 96 well ELISA plates with 0.5 µg per well 12CA5 anti-HA antibody in 100 µl PBS. Store overnight at 4°C.
2. Remove unbound HA antibody from wells by inverting plate. Wash plate with dH₂O. Pat the plate on a paper towel to remove excess liquid.
- 15 3. Add 150 µl Blocking Buffer to each well. Incubate, with shaking, for 30 min at room temperature.
4. Wash plate 4x with TBS-T.
5. Dilute lysate in PBS (1.5 µg lysate/100 µl PBS).
- 20 6. Add 100 µl of diluted lysate to each well. Shake at room temperature for 1 hr.
7. Wash as in step 4.
8. Add 50 µl of 2X kinase Buffer to ELISA plate containing captured pyk2-HA.
- 25 9. Add 25 µL of 400 µM test compound in 4% DMSO to each well. For control wells use 4% DMSO alone.
10. Add 25 µL of 0.5 M EDTA to negative control wells.
11. Add 25 µl of 20 µM ATP to all wells. Incubate, with shaking, for 10 minutes.
- 30 12. Stop reaction by adding 25 µl 500 mM EDTA (pH 8.0) to all wells.
13. Wash as in step 4.
14. Add 100 µL HRP conjugated anti-Ptyr diluted 1:6000 in Antibody Dilution Buffer to each well. Incubate,

- with shaking, for 1 hr. at room temperature.
15. Wash plate 3X with TBST and 1X with PBS.
 16. Add 100 μ L of ABST solution to each well.
 17. If necessary, stop the development reaction by
5 adding 20 μ L 10% SDS to each well.
 18. Read plate on ELISA reader with test filter at 410
nM and reference filter at 630 nM.

FGFR1 BIOASSAY

10 This assay is used to measure the in vitro kinase
activity of FGF1-R in an ELISA assay.

Materials and Reagents:

1. Costar 96-well Elisa plates (Corning Catalog # 3369).
- 15 2. Poly(Glu-Tyr) (Sigma Catalog # PO275).
3. PBS (Gibco Catalog # 450-1300EB)
4. 50 mM Hepes Buffer Solution.
5. Blocking Buffer (5% BSA/PBS).
6. Purified GST-FGFR1 (SUGEN, Inc.)
- 20 7. Kinase Dilution Buffer.
Mix 500 μ l 1M Hepes (GIBCO), 20 μ l 5% BSA/PBS, 10 μ l
100mM sodium orthovanadate and 50 μ l 5M NaCl.
8. 10mM ATP
9. ATP/MnCl₂ phosphorylation mix: mix 20 μ L ATP, 400 μ L
25 1M MnCl₂ and 9.56 ml dH₂O.
10. NUNC 96-well V bottom polypropylene plates (Applied
Scientific Catalog # AS-72092).
11. 0.5M EDTA.
12. 0.05% TBST
- 30 Add 500 μ L TWEEN to 1 liter TBS.
13. Rabbit polyclonal anti-phosphotyrosine serum (SUGEN,
Inc.).
14. Goat anti-rabbit IgG peroxidase conjugate
(Biosource, Catalog # ALI0404).

15. ABTS Solution.

16. ABTS/H₂O₂ solution.

Procedure:

1. Coat Costar 96 well ELISA plates with 1 µg per well Poly(Glu-Tyr) in 100µl PBS. Store overnight at 4° C.
2. Wash coated plates once with PBS.
3. Add 150 µL of 5%BSA/PBS Blocking Buffer to each well. Incubate, with shaking, for 1 hr at room temperature.
4. Wash plate 2x with PBS, then once with 50mM Hepes. Pat plates on a paper towel to remove excess liquid and bubbles.
5. Add 25µL of 0.4 mM test compound in 4% DMSO or 4% DMSO alone (controls) to plate.
6. Dilute purified GST-FGFR1 in Kinase Dilution Buffer (5 ng kinase/50ul KDB/well).
7. Add 50µL of diluted kinase to each well.
8. Start kinase reaction by adding 25µl/well of freshly prepared ATP/Mn++ (0.4 ml 1M MnCl₂, 40 µL 10 mM ATP, 9.56 ml dH₂O), freshly prepared).
9. Stop reaction with 25µL of 0.5M EDTA.
10. Wash plate 4x with fresh TBST.
11. Make up Antibody Dilution Buffer: For 50 ml, mix 5 ml of 5% BSA, 250 µl of 5% milk and 50 µl of 100mM sodium vanadate, bring to final volume with 0.05% TBST.
12. Add 100 µl per well of anti-phosphotyrosine (1:10000 dilution in ADB). Incubate, with shaking for 1 hr. at room temperature.
13. Wash as in step 10.
14. Add 100 µl per well of Biosource Goat anti-rabbit IgG peroxidase conjugate (1:6000 dilution in ADB). Incubate, with shaking for 1 hr. at room temperature.

15. Wash as in step 10 and then with PBS to remove bubbles and excess TWEEN.
16. Add 100 µl of ABTS/H₂O₂ solution to each well.
17. Incubate, with shaking, for 10 to 20 minutes.
- 5 Remove any bubbles.
18. Read assay on Dynatech MR7000 elisa reader: test filter at 410 nM, reference filter at 630 nM.

EGFR BIOASSAY

10 This assay is used to the in vitro kinase activity of EGFR in an ELISA assay.

Materials and Reagents:

1. Corning 96-well Elisa plates.
2. SUMO1 monoclonal anti-EGFR antibody (SUGEN, Inc.).
- 15 3. PBS.
4. TBST Buffer.
5. Blocking Buffer: for 100 ml, mix 5.0 g Carnation® Instant Non-fat Milk with 100 ml of PBS.
6. A431 cell lysate (SUGEN, Inc.).
- 20 7. TBS Buffer.
8. TBS + 10% DMSO: for 1L, mix 1.514 g TRIS, 2.192 g NaCl and 25 ml DMSO; bring to 1 liter total volume with dH₂O.
9. ATP (Adenosine-5'-triphosphate, from Equine muscle, Sigma Cat. No. A-5394), 1.0 mM solution in dH₂O.
- 25 This reagent should be made up immediately prior to use and kept on ice.
10. 1.0 mM MnCl₂.
11. ATP/MnCl₂ phosphorylation mix: for 10 ml, mix 300 µl
- 30 of 1 mM ATP, 500 µl MnCl₂ and 9.2 ml dH₂O. Prepare just prior to use, keep on ice.
12. NUNC 96-well V bottom polypropylene plates.
13. EDTA.

14. Rabbit polyclonal anti-phosphotyrosine serum (SUGEN, Inc.).
15. Goat anti-rabbit IgG peroxidase conjugate (Biosource Cat. No. ALI0404).
- 5 16. ABTS.
17. 30% Hydrogen peroxide.
18. ABTS/H₂O₂.
19. 0.2 M HCl.

Procedure:

- 10 1. Coat Corning 96 well ELISA plates with 0.5 µg SUMO1 in 100 µl PBS per well, hold overnight at 4° C.
2. Remove unbound SUMO1 from wells by inverting plate to remove liquid. Wash 1x with dH₂O. Pat the plate on a paper towel to remove excess liquid.
- 15 3. Add 150 µl of Blocking Buffer to each well. Incubate, with shaking, for 30 min. at room temperature.
4. Wash plate 3x with deionized water, then once with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.
- 20 5. Dilute lysate in PBS (7 µg lysate/100 µl PBS).
6. Add 100 µl of diluted lysate to each well. Shake at room temperature for 1 hr.
7. Wash plates as in 4, above.
- 25 8. Add 120 µl TBS to ELISA plate containing captured EGFR.
9. Dilute test compound 1:10 in TBS, place in well
10. Add 13.5 µl diluted test compound to ELISA plate. To control wells, add 13.5 µl TBS in 10% DMSO.
- 30 11. Incubate, with shaking, for 30 minutes at room temperature.
12. Add 15 µl phosphorylation mix to all wells except negative control well. Final well volume should be approximately 150 µl with 3 µM ATP/5 mM MnCl₂ final

concentration in each well. Incubate with shaking for 5 minutes.

13. Stop reaction by adding 16.5 μ l of EDTA solution while shaking. Shake for additional 1 min.
- 5 14. Wash 4x with deionized water, 2x with TBST.
15. Add 100 μ l anti-phosphotyrosine (1:3000 dilution in TBST) per well. Incubate, with shaking, for 30-45 min. at room temperature.
16. Wash as in 4, above.
- 10 17. Add 100 μ l Biosource Goat anti-rabbit IgG peroxidase conjugate (1:2000 dilution in TBST) to each well. Incubate with shaking for 30 min. at room temperature.
18. Wash as in 4, above.
- 15 19. Add 100 μ l of ABTS/H₂O₂ solution to each well.
20. Incubate 5 to 10 minutes with shaking. Remove any bubbles.
21. If necessary, stop reaction by adding 100 μ l 0.2 M HCl per well.
- 20 22. Read assay on Dynatech MR7000 ELISA reader: test filter at 410 nM, reference filter at 630 nM.

PDGFR BIOASSAY

This assay is used to the in vitro kinase activity of PDGFR in an ELISA assay.

Materials and Reagents:

1. Corning 96-well Elisa plates
2. 28D4C10 monoclonal anti-PDGFR antibody (SUGEN, Inc.).
3. PBS.
- 30 4. TBST Buffer.
5. Blocking Buffer (same as for EGFR bioassay).
6. PDGFR- β expressing NIH 3T3 cell lysate (SUGEN, Inc.).
7. TBS Buffer.

8. TBS + 10% DMSO.
9. ATP.
10. $MnCl_2$.
- 5 11. Kinase buffer phosphorylation mix: for 10 ml, mix
250 μ l 1M TRIS, 200 μ l 5M NaCl, 100 μ l 1M $MnCl_2$ and
50 μ l 100 mM Triton X-100 in enough dH_2O to make 10
ml.
12. NUNC 96-well V bottom polypropylene plates.
13. EDTA.
- 10 14. Rabbit polyclonal anti-phosphotyrosine serum
(SUGEN, Inc.).
15. Goat anti-rabbit IgG peroxidase conjugate (Biosource
Cat. No. ALI0404).
16. ABTS.
- 15 17. Hydrogen peroxide, 30% solution.
18. ABTS/ H_2O_2 .
19. 0.2 M HCl.

Procedure:

- 20 1. Coat Corning 96 well ELISA plates with 0.5 μ g
28D4C10 in 100 μ l PBS per well, hold overnight at 4°
C.
2. Remove unbound 28D4C10 from wells by inverting plate
to remove liquid. Wash 1x with dH_2O . Pat the plate on
a paper towel to remove excess liquid.
- 25 3. Add 150 μ l of Blocking Buffer to each well. Incubate
for 30 min. at room temperature with shaking.
4. Wash plate 3x with deionized water, then once with
TBST. Pat plate on a paper towel to remove excess
liquid and bubbles.
- 30 5. Dilute lysate in HNTG (10 μ g lysate/100 μ l HNTG).
6. Add 100 μ l of diluted lysate to each well. Shake at
room temperature for 60 min.
7. Wash plates as described in Step 4.
8. Add 80 μ l working kinase buffer mix to ELISA plate

containing captured PDGFR.

9. Dilute test compound 1:10 in TBS in 96-well polypropylene plates.
10. Add 10 µl diluted test compound to ELISA plate. To control wells, add 10 µl TBS + 10% DMSO. Incubate with shaking for 30 minutes at room temperature.
11. Add 10 µl ATP directly to all wells except negative control well (final well volume should be approximately 100 µl with 20 µM ATP in each well.) Incubate 30 minutes with shaking.
12. Stop reaction by adding 10 µl of EDTA solution to each well.
13. Wash 4x with deionized water, twice with TBST.
14. Add 100 µl anti-phosphotyrosine (1:3000 dilution in TBST) per well. Incubate with shaking for 30-45 min. at room temperature.
15. Wash as in Step 4.
16. Add 100 µl Biosource Goat anti-rabbit IgG peroxidase conjugate (1:2000 dilution in TBST) to each well. Incubate with shaking for 30 min. at room temperature.
17. Wash as in Step 4.
18. Add 100 µl of ABTS/H₂O₂ solution to each well.
19. Incubate 10 to 30 minutes with shaking. Remove any bubbles.
20. If necessary stop reaction with the addition of 100 µl 0.2 M HCl per well.
21. Read assay on Dynatech MR7000 ELISA reader with test filter at 410 nm and reference filter at 630 nm.

CELLULAR HER-2 KINASE ASSAY

This assay is used to measure HER-2 kinase activity in whole cells in an ELISA format.

Materials and Reagents:

1. DMEM (GIBCO Catalog #11965-092).

2. Fetal Bovine Serum (FBS, GIBCO Catalog #16000-044), heat inactivated in a water bath for 30 min. at 56° C.
3. Trypsin (GIBCO Catalog #25200-056).
- 5 4. L-Glutamine (GIBCO Catalog #25030-081).
- 5 5. HEPES (GIBCO Catalog #15630-080).
- 6 6. Growth Media: Mix 500 ml DMEM, 55 ml heat inactivated FBS, 10 ml HEPES and 5.5 ml L-Glutamine.
- 7 7. Starve Media: Mix 500 ml DMEM, 2.5 ml heat inactivated FBS, 10 ml HEPES and 5.5 ml L-Glutamine.
- 10 8. PBS.
- 9 9. Flat Bottom 96-well Tissue Culture Micro Titer Plates (Corning Catalog # 25860).
- 10 10. 15 cm Tissue Culture Dishes (Corning Catalog #08757148).
- 15 11. Corning 96-well ELISA Plates.
- 12 12. NUNC 96-well V bottom polypropylene plates.
- 13 13. Costar Transfer Cartridges for the Transtar 96 (Costar Catalog #7610).
- 20 14. SUMO 1: monoclonal anti-EGFR antibody (SUGEN, Inc.).
- 15 15. TBST Buffer.
- 16 16. Blocking Buffer : 5% Carnation Instant Milk® in PBS.
- 17 17. EGF Ligand: EGF-201, Shinko American, Japan.
- 25 Suspend powder in 100 uL of 10mM HCl. Add 100uL 10mM NaOH. Add 800 uL PBS and transfer to an Eppendorf tube, store at -20°C until ready to use.
- 18 18. HNTG Lysis Buffer: For Stock 5X HNTG, mix 23.83 g Hepes, 43.83 g NaCl, 500 ml glycerol and 100 ml Triton X-100 and enough dH₂O to make 1 L of total solution.
- 30

For 1X HNTG*, mix 2 ml 5X HNTG, 100 µL 0.1M Na₃VO₄, 250 µL 0.2M Na₄P₂O₇ and 100 µL EDTA.

19. EDTA.
20. Na_3VO_4 : To make stock solution, mix 1.84 g Na_3VO_4 with 90 ml dH_2O . Adjust pH to 10. Boil in microwave for one minute (solution becomes clear). Cool to room temperature. Adjust pH to 10. Repeat heating/cooling cycle until pH remains at 10.
21. 200 mM $\text{Na}_4\text{P}_2\text{O}_7$.
22. Rabbit polyclonal antiserum specific for phosphotyrosine (anti-Ptyr antibody, SUGEN, Inc.).
23. Affinity purified antiserum, goat anti-rabbit IgG antibody, peroxidase conjugate (Biosource Cat # ALI0404).
24. ABTS Solution.
25. 30 % Hydrogen peroxide solution.
26. ABTS/ H_2O_2 .
27. 0.2 M HCl.

Procedure:

1. Coat Corning 96 well ELISA plates with SUMO1 at 1.0 ug per well in PBS, 100 ul final volume/well. Store overnight at 4°C.
2. On day of use, remove coating buffer and wash plate 3 times with dH_2O and once with TBST buffer. All washes in this assay should be done in this manner, unless otherwise specified.
3. Add 100 ul of Blocking Buffer to each well. Incubate plate, with shaking, for 30 min. at room temperature. Just prior to use, wash plate.
4. Use EGFr/HER-2 chimera/3T3-C7 cell line for this assay.
5. Choose dishes having 80-90 % confluence. Collect cells by trypsinization and centrifuge at 1000 rpm at room temperature for 5 min.
6. Resuspend cells in starve medium and count with trypan blue. Viability above 90% is required. Seed cells in starve medium at a density of 2,500 cells

per well, 90 ul per well, in a 96 well microtiter plate. Incubate seeded cells overnight at 37° under 5% CO₂.

7. Start the assay two days after seeding.
- 5 8. Test compounds are dissolved in 4% DMSO. Samples are then further diluted directly on plates with starve-DMEM. Typically, this dilution will be 1:10 or greater. All wells are then transferred to the cell plate at a further 1:10 dilution (10µl sample and media into 90 µl of starve media). The final DMSO concentration should be 1% or lower. A standard serial dilution may also be used.
- 10 9. Incubate under 5% CO₂ at 37°C for 2 hours.
- 15 10. Prepare EGF ligand by diluting stock EGF (16.3 uM) in warm DMEM to 150 nM.
11. Prepare fresh HNTG* sufficient for 100 ul per well; place on ice.
12. After 2 hour incubation with test compound, add prepared EGF ligand to cells, 50 ul per well, for a final concentration of 50 nM. Positive control wells receive the same amount of EGF. Negative controls do not receive EGF. Incubate at 37° C for 10 min.
- 20 13. Remove test compound, EGF, and DMEM. Wash cells once with PBS.
- 25 14. Transfer HNTG* to cells, 100 ul per well. Place on ice for 5 minutes. Meanwhile, remove blocking buffer from ELISA plate and wash.
15. Scrape cells from plate with a micropipettor and homogenize cell material by repeatedly aspirating and dispensing the HNTG* lysis buffer. Transfer lysate to a coated, blocked, washed ELISA plate.
- 30 16. Incubate, with shaking, at room temperature for 1 hr.

17. Remove lysate, wash. Transfer freshly diluted anti-Ptyr antibody (1:3000 in TBST) to ELISA plate, 100 ul per well.
18. Incubate, with shaking, at room temperature, for 30 min.
19. Remove anti-Ptyr antibody, wash. Transfer freshly diluted BIOSOURCE antibody to ELISA plate(1:8000 in TBST, 100 ul per well).
20. Incubate, with shaking, at room temperature for 30 min.
21. Remove BIOSOURCE antibody, wash. Transfer freshly prepared ABTS/H₂O₂ solution to ELISA plate, 100 ul per well.
22. Incubate, with shaking, for 5-10 minutes. Remove any bubbles.
23. Stop reaction by adding 100 ul of 0.2M HCl per well.
24. Read assay on Dynatech MR7000 ELISA reader with test filter set at 410 nM and reference filter at 630 nM.

CDK2/CYCLIN A ASSAY

This assay is used to measure the in vitro serine/threonine kinase activity of human cdk2/cyclin A in a Scintillation Proximity Assay (SPA).

Materials and Reagents.

1. Wallac 96-well polyethylene terephthalate (flexi) plates (Wallac Catalog # 1450-401).
2. Amersham Redivue [$\gamma^{33}\text{P}$] ATP (Amersham catalog #AH 9968).
3. Amersham streptavidin coated polyvinyltoluene SPA beads (Amersham catalog #RPNQ0007). The beads should be reconstituted in PBS without magnesium or calcium, at 20 mg/ml.
4. Activated cdk2/cyclin A enzyme complex purified from Sf9 cells (SUGEN, Inc.).

5. Biotinylated peptide substrate (Debtide). Peptide biotin-X-PKTPKKAKKL is dissolved in dH₂O at a concentration of 5 mg/ml.
6. 20% DMSO in dH₂O.
- 5 7. Kinase buffer: for 10 ml, mix 9.1 ml dH₂O, 0.5 ml TRIS(pH 7.4), 0.2 ml 1M MgCl₂, 0.2 ml 10% NP40 and 0.02 ml 1M DTT, added fresh just prior to use.
8. 10 mM ATP in dH₂O.
9. 1M Tris, pH adjusted to 7.4 with HCl.
- 10 10. 1M MgCl₂.
11. 1M DTT.
12. PBS (Gibco Catalog # 14190-144).
13. 0.5M EDTA.
14. Stop solution: For 10 ml, mix 9.25 ml PBS, 0.05 ml 10 mM ATP, 0.1 ml 0.5 M EDTA, 0.1 ml 10% Triton X-100 and 1.5 ml of 50 mg/ml SPA beads.
- 15

Procedure:

1. Prepare solutions of test compounds at 4x the desired final concentration in 5% DMSO. Add 10 ul to each well. For positive and negative controls, use 10 ul 20% DMSO alone in wells.
- 20 2. Dilute the peptide substrate (deb-tide) 1:250 with dH₂O to give a final concentration of 0.02 mg/ml.
3. Mix 24 ul 0.1 mM ATP with 24 uCi $\gamma^{33}\text{P}$ ATP and enough dH₂O to make 600 ul.
- 25 4. Mix diluted peptide and ATP solutions 1:1 (600 ul + 600 ul per plate). Add 10 ul of this solution to each well.
5. Dilute 5 ul of cdk2/cyclin A solution into 2.1 ml 2x kinase buffer (per plate). Add 20 ul enzyme per well. For negative controls, add 20 ul 2x kinase buffer without enzyme.
- 30 6. Mix briefly on a plate shaker; incubate for 60 minutes.
- 35 7. Add 200 ul stop solution per well.

8. Let stand at least 10 min.
9. Spin plate at approx. 2300 rpm for 10-15 min.
10. Count plate on Trilux reader.

MET TRANSPHOSPHORYLATION ASSAY

5 This assay is used to measure phosphotyrosine levels on a poly(glutamic acid:tyrosine, 4:1) substrate as a means for identifying agonists/antagonists of met transphosphorylation of the substrate.

Materials and Reagents:

- 10 1. Corning 96-well Elisa plates, Corning Catalog # 25805-96.
2. Poly(glu-tyr), 4:1, Sigma, Cat. No; P 0275.
3. PBS, Gibco Catalog # 450-1300EB
4. 50 mM HEPES
- 15 5. Blocking Buffer: Dissolve 25 g Bovine Serum Albumin, Sigma Cat. No A-7888, in 500 ml PBS, filter through a 4 µm filter.
6. Purified GST fusion protein containing the Met kinase domain, Sugen, Inc.
- 20 7. TBST Buffer.
8. 10% aqueous (MilliQue H₂O) DMSO.
9. 10 mM aqueous (dH₂O) Adenosine-5'-triphosphate, Sigma Cat. No. A-5394.
10. 2X Kinase Dilution Buffer: for 100 ml, mix 10 mL 1M HEPES at pH 7.5 with 0.4 mL 5% BSA/PBS, 0.2 mL 0.1 M sodium orthovanadate and 1 mL 5M sodium chloride in 88.4 mL dH₂O.
- 25 11. 4X ATP Reaction Mixture: for 10 mL, mix 0.4 mL 1 M manganese chloride and 0.02 mL 0.1 M ATP in 9.56 mL dH₂O.
- 30 12. 4X Negative Controls Mixture: for 10 mL, mix 0.4 mL 1 M manganese chloride in 9.6 mL dH₂O.
13. NUNC 96-well V bottom polypropylene plates, Applied Scientific Catalog # S-72092
- 35 14. 500 mM EDTA.

15. Antibody Dilution Buffer: for 100 mL, mix 10 mL 5% BSA/PBS, 0.5 mL 5% Carnation® Instant Milk in PBS and 0.1 mL 0.1 M sodium orthovanadate in 88.4 mL TBST.
- 5 16. Rabbit polyclonal antophosphotyrosine antibody, Sugen, Inc.
17. Goat anti-rabbit horseradish peroxidase conjugated antibody, Biosource, Inc.
18. ABTS Solution: for 1 L, mix 19.21 g citric acid, 35.49 g Na_2HPO_4 and 500 mg ABTS with sufficient dH_2O to make 1 L.
- 10 19. ABTS/ H_2O_2 : mix 15 mL ABST solution with 2 μL H_2O_2 five minutes before use.
20. 0.2 M HCl

15
Procedure:

1. Coat ELISA plates with 2 μg Poly(Glu-Tyr) in 100 μL PBS, hold overnight at 4 ° C.
- 20 2. Block plate with 150 μL of 5% BSA/PBS for 60 min.
3. Wash plate twice with PBS then once with 50 mM Hepes buffer pH 7.4.
4. Add 50 μL of the diluted kinase to all wells.
(Purified kinase is diluted with Kinase Dilution Buffer. Final concentration should be 10 ng/well.)
- 25 5. Add 25 μL of the test compound (in 4% DMSO) or DMSO alone (4% in dH_2O) for controls to plate.
6. Incubate the kinase/compound mixture for 15 minutes.
7. Add 25 μL of 40 mM MnCl_2 to the negative control wells.
- 30 8. Add 25 μL ATP/ MnCl_2 mixture to the all other wells (except the negative controls). Incubate for 5 min.
9. Add 25 μL 500 mM EDTA to stop reaction.
10. Wash plate 3x with TBST.

11. Add 100 μ L rabbit polyclonal anti-Ptyr diluted 1:10,000 in Antibody Dilution Buffer to each well. Incubate, with shaking, at room temperature for one hour.
- 5 12. Wash plate 3x with TBST.
13. Dilute Biosource HRP conjugated anti-rabbit antibody 1: 6,000 in Antibody Dilution buffer. Add 100 μ L per well and incubate at room temperature, with shaking, for one hour.
- 10 14. Wash plate 1X with PBS.
15. Add 100 μ l of ABTS/H₂O₂ solution to each well.
16. If necessary, stop the development reaction with the addition of 100 μ l of 0.2M HCl per well.
17. Read plate on Dynatech MR7000 elisa reader with the test filter at 410 nM and the reference filter at 630 nM.

IGF-1 TRANSPHOSPHORYLATION ASSAY

This assay is used to measure the phosphotyrosine level in poly(glutamic acid:tyrosine, 4:1) for the identification of agonists/antagonists of gst-IGF-1 transphosphorylation of a substrate.

Materials and Reagents:

1. Corning 96-well Elisa plates.
2. Poly(Glu-tyr), 4:1, Sigma Cat. No. P 0275.
- 25 3. PBS, Gibco Catalog # 450-1300EB.
4. 50 mM HEPES
5. TBB Blocking Buffer: for 1 L, mix 100 g BSA, 12.1 g TRIS (pH 7.5), 58.44 g sodium chloride and 10 mL 1%TWEEN-20.
- 30 6. Purified GST fusion protein containing the IGF-1 kinase domain (Sugen, Inc.)
7. TBST Buffer: for 1 L, mix 6.057 g Tris, 8.766 g sodium chloride and 0.5 ml TWEEN-20 with enough dH₂O to make 1 liter.

8. 4% DMSO in Milli-Q H₂O.
9. 10 mM ATP in dH₂O.
10. 2X Kinase Dilution Buffer: for 100 mL, mix 10 mL 1 M HEPES (pH 7.5), 0.4 mL 5% BSA in dH₂O, 0.2 mL 0.1 M sodium orthovanadate and 1 mL 5 M sodium chloride with enough dH₂O to make 100 mL.
11. 4X ATP Reaction Mixture: for 10 mL, mix 0.4 mL 1 M MnCl₂ and 0.008 mL 0.01 M ATP and 9.56 mL dH₂O.
12. 4X Negative Controls Mixture: mix 0.4 mL 1 M MnCl₂ in 9.60 mL dH₂O.
13. NUNC 96-well V bottom polypropylene plates.
14. 500 mM EDTA in dH₂O.
15. Antibody Dilution Buffer: for 100 mL, mix 10 mL 5% BSA in PBS, 0.5 mL 5% Carnation Instant Non-fat Milk in PBS and 0.1 mL 0.1 M sodium orthovanadate in 88.4 mL TBST.
16. Rabbit Polyclonal antiphosphotyrosine antibody, Sugan, Inc.
17. Goat anti-rabbit HRP conjugated antibody, Biosource.
18. ABTS Solution.
20. ABTS/H₂O₂: mix 15 mL ABTS with 2 µL H₂O₂ 5 minutes before using.
21. 0.2 M HCl in dH₂O.

Procedure:

1. Coat ELISA plate with 2.0 µg / well Poly(Glu, Tyr), 4:1 (Sigma P0275) in 100 µl PBS. Store plate overnight at 4° C.
2. Wash plate once with PBS.
3. Add 100 µl of TBB Blocking Buffer to each well. Incubate plate for 1 hour with shaking at room temperature.
4. Wash plate once with PBS, then twice with 50 mM Hepes buffer pH 7.5.
5. Add 25 µL of test compound in 4% DMSO (obtained by

diluting a stock solution of 10 mM test compound in 100% DMSO with dH₂O) to plate.

6. Add 10.0 ng of gst-IGF-1 kinase in 50 µl Kinase Dilution Buffer to all wells.
- 5 7. Start kinase reaction by adding 25µl 4X ATP Reaction Mixture to all test wells and positive control wells. Add 25µl 4X Negative Controls Mixture to all negative control wells. Incubates for 10 minutes, with shaking, at room temperature.
- 10 8. Add 25µl 0.5M EDTA (pH 8.0) to all wells.
9. Wash plate 4x with TBST Buffer.
10. Add rabbit polyclonal anti-phosphotyrosine antisera at a dilution of 1:10,000 in 100µl Antibody Dilution Buffer to all wells. Incubate, with shaking, at room temperature for 1 hour.
- 15 11. Wash plate as in step 9.
12. Add 100 µL Biosource anti-rabbit HRP at a dilution of 1:10,000 in Antibody dilution buffer to all wells. Incubate, with shaking, at room temperature for 1 hour.
- 20 13. Wash plate as in step 9, follow with one wash with PBS to remove bubbles and excess Tween-20.
14. Develop by adding 100µl/well ABTS/H₂O₂ to each well.
15. After about 5 minutes, read on ELISA reader with test filter at 410 nm and referenced filter at 630 nm.
- 25

BRDU INCORPORATION ASSAYS

The following assays use cells engineered to express a selected receptor and then evaluate the effect of a compound of interest on the activity of ligand-induced DNA synthesis by determining BrdU incorporation into the DNA.

The following materials, reagents and procedure are general to each of the following BrdU incorporation assays. Variances in specific assays are noted.

General Materials and Reagents:

1. The appropriate ligand.
2. The appropriate engineered cells.
3. BrdU Labeling Reagent: 10 mM, in PBS, pH7.4 (Roche Molecular Biochemicals, Indianapolis, IN).
4. FixDenat: fixation solution (Roche Molecular Biochemicals, Indianapolis, IN).
5. Anti-BrdU-POD: mouse monoclonal antibody conjugated with peroxidase (Chemicon, Temecula, CA).
6. TMB Substrate Solution: tetramethylbenzidine (TMB, ready to use, Roche Molecular Biochemicals, Indianapolis, IN).
7. PBS Washing Solution : 1X PBS, pH 7.4.
8. Albumin, Bovine (BSA), fraction V powder (Sigma Chemical Co., USA).

General Procedure:

1. Cells are seeded at 8000 cells/well in 10% CS, 2mM Gln in DMEM, in a 96 well plate. Cells are incubated overnight at 37°C in 5% CO₂.
2. After 24 hours, the cells are washed with PBS, and then are serum-starved in serum free medium (0%CS DMEM with 0.1% BSA) for 24 hours.
3. On day 3, the appropriate ligand and the test compound are added to the cells simultaneously. The negative control wells receive serum free DMEM with 0.1% BSA only; the positive control cells receive the ligand but no test compound. Test compounds are prepared in serum free DMEM with ligand in a 96 well plate, and serially diluted for 7 test concentrations.
4. After 18 hours of ligand activation, diluted BrdU labeling reagent (1:100 in DMEM, 0.1% BSA) is added and the cells are incubated with BrdU (final concentration is 10 µM) for 1.5 hours.
5. After incubation with labeling reagent, the medium

- is removed by decanting and tapping the inverted plate on a paper towel. FixDenat solution is added (50 μ l/well) and the plates are incubated at room temperature for 45 minutes on a plate shaker.
6. The FixDenat solution is removed by decanting and tapping the inverted plate on a paper towel. Milk is added (5% dehydrated milk in PBS, 200 μ l/well) as a blocking solution and the plate is incubated for 30 minutes at room temperature on a plate shaker.
7. The blocking solution is removed by decanting and the wells are washed once with PBS. Anti-BrdU-POD solution is added (1:200 dilution in PBS, 1% BSA, 50 μ l/well) and the plate is incubated for 90 minutes at room temperature on a plate shaker.
8. The antibody conjugate is removed by decanting and rinsing the wells 5 times with PBS, and the plate is dried by inverting and tapping on a paper towel.
9. TMB substrate solution is added (100 μ l/well) and incubated for 20 minutes at room temperature on a plate shaker until color development is sufficient for photometric detection.
10. The absorbance of the samples are measured at 410 nm (in "dual wavelength" mode with a filter reading at 490 nm, as a reference wavelength) on a Dynatech ELISA plate reader.

EGF-Induced BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
2. 3T3/EGFRc7.

EGF-Induced Her-2-driven BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
2. 3T3/EGFr/Her2/EGFr (EGFr with a Her-2 kinase domain).

Remaining Materials and Reagents and Procedure, as above.

EGF-Induced Her-4-driven BrdU Incorporation Assay

Materials and Reagents:

1. Mouse EGF, 201 (Toyobo Co., Ltd., Japan).
- 5 2. 3T3/EGFr/Her4/EGFr (EGFr with a Her-4 kinase domain).

Remaining Materials and Reagents and Procedure, as above.

PDGF-Induced BrdU Incorporation Assay

Materials and Reagents:

- 10 1. Human PDGF B/B (Boehringer Mannheim, Germany).
2. 3T3/EGFRc7.

Remaining Materials and Reagents and Procedure, as above.

FGF-Induced BrdU Incorporation Assay

Materials and Reagents:

- 15 1. Human FGF2/bFGF (Gibco BRL, USA).
2. 3T3c7/EGFr

Remaining Materials and Reagents and Procedure, as above.

IGF1-Induced BrdU Incorporation Assay

Materials and Reagents:

- 20 1. Human, recombinant (G511, Promega Corp., USA)
2. 3T3/IGF1r.

Remaining Materials and Reagents and Procedure, as above.

Insulin-Induced BrdU Incorporation Assay

Materials and Reagents:

- 25 1. Insulin, crystalline, bovine, Zinc (13007, Gibco BRL, USA).
2. 3T3/H25.

Remaining Materials and Reagents and Procedure, as above.

HGF-Induced BrdU Incorporation Assay

30 Materials and Reagents:

1. Recombinant human HGF (Cat. No. 249-HG, R&D Systems, Inc. USA).
2. BxPC-3 cells (ATCC CRL-1687).

Remaining Materials and Reagents, as above.

Procedure:

1. Cells are seeded at 9000 cells/well in RPMI 10% FBS in a 96 well plate. Cells are incubated overnight at 37°C in 5% CO₂.
- 5 2. After 24 hours, the cells are washed with PBS, and then are serum starved in 100 µl serum-free medium (RPMI with 0.1% BSA) for 24 hours.
3. On day 3, 25 µl containing ligand (prepared at 1 µg/ml in RPMI with 0.1% BSA; final HGF conc. is 200
10 ng/ml) and test compounds are added to the cells. The negative control wells receive 25 µl serum-free RPMI with 0.1% BSA only; the positive control cells receive the ligand (HGF) but no test compound. Test compounds are prepared at 5 times their final
15 concentration in serum-free RPMI with ligand in a 96 well plate, and serially diluted to give 7 test concentrations. Typically, the highest final concentration of test compound is 100 µM, and 1:3 dilutions are used (i.e. final test compound
20 concentration range is 0.137-100 µM).
4. After 18 hours of ligand activation, 12.5 µl of diluted BrdU labeling reagent (1:100 in RPMI, 0.1% BSA) is added to each well and the cells are
25 incubated with BrdU (final concentration is 10 µM) for 1 hour.
5. Same as General Procedure.
6. Same as General Procedure.
7. The blocking solution is removed by decanting and
the wells are washed once with PBS. Anti-BrdU-POD
30 solution (1:100 dilution in PBS, 1% BSA) is added (100 µl/well) and the plate is incubated for 90 minutes at
room temperature on a plate shaker.
8. Same as General Procedure.
9. Same as General Procedure.

10. Same as General Procedure.

EXPONENTIAL BrdU INCORPORATION ASSAY

This assay is used to measure the proliferation (DNA synthesis) of exponentially growing A431 cells. The assay will screen for compounds that inhibit cell cycle progression.

Materials and Reagents:

Healthy growing A431 cells. The remainder of the Materials and Reagents are the same as listed above in the general protocol section.

Procedure:

1. A431 cells are seeded at 8000 cells/well in 10% FBS, 2mM Gln in DMEM, on a 96-well plate. Cells are incubated overnight at 37° C in 5% CO₂.

2. On day 2, test compounds are serially diluted to 7 test concentrations in the same growth medium on a 96-well plate and then are added to the cells on a 96-well tissue culture plate.

3. After 20 - 24 hours of incubation, diluted BrdU labeling reagent (1:100 in DMEM, 0.1% BSA) is added and the cells are incubated with BrdU (final concentration is 10 µM) for 2 hours.

Steps 5 - 10 of the General Procedure are used to complete the assay.

ZenSrc ASSAY

This assay is used to screen for inhibitors of the tyrosine kinase Src.

Materials and Reagents:

1. Coating buffer: PBS containing sodium azide (0.2mg/ml).

2. 1% w/v BSA in PBS.

3. Wash buffer: PBS containing 0.05% v/v Tween 20 (PBS-TWEEN)

4. 500mM HEPES pH7.4.
5. ATP (40 μ M) + MgCl₂ (80mM) in distilled water.
6. MgCl₂ (80mM) in distilled water (for no ATP blanks).
7. Test compounds, 10mM in DMSO.
- 5 8. Assay Buffer: 100 mM HEPES, pH 7.4, containing 2mM
DTT, 0.2mM sodium orthovanadate and 0.2mgs/ml BSA.
9. Partially purified recombinant human Src (UBI (14-
117)
- 10 10. Anti-phosphotyrosine (SUGEN rabbit polyclonal anti-
PY).
11. HRP-linked goat anti-rabbit Ig (Biosource
International #6430)
12. HRP substrate ABTS or Pierce Peroxidase substrate.
- 15 13. Corning ELISA plates.

Procedure:

1. Coat plates with 100 μ l of 20 μ g/ml poly(Glu-Tyr)
(Sigma Cat. No.P0275) containing 0.01% sodium azide. Hold
overnight at 4° C.
- 20 2. Block with 1% BSA at 100 μ l/well for one hour at room
temperature.
3. Plate test compounds (10mM in DMSO) at 2 μ l/well on a
Costar plate ready for dilution with dH₂O and plating to
reaction plates.
- 25 4. Dilute Src kinase 1:10,000 in Reaction Buffer, for 5
plates prepare 25 ml as follows: 2.5mls 1M HEPES pH7.4 (stored
sterile at 4°C), 21.85 ml distilled water, 0.1 ml 5% BSA, 0.5
ml 10 mM sodium orthovanadate (stored sterile at 4°C), 50 μ l
1.0M DTT (stored frozen at -20°C), and 2.5 μ l Src Kinase
30 (stored frozen at -80°C).
5. Add 48 μ l of distilled water to the 2 μ l of each
compound in the dilution plate then add 25 μ l/well of this to
the reaction plate.
6. Add 50 μ l of HRP to each reaction buffer well and

then 25µl ATP-MgCl₂/well (MgCl₂ only to no ATP blanks).
Incubate at room temperature for 15 minutes on plate shaker.
Stop reaction by adding 25µl of 0.5M EDTA to each well.

7. Wash 4X with PBS-TWEEN.

5 8. Add 100µl anti-phosphotyrosine (1:10,000 of anti-pTyr serum or 1:3,000 of 10% glycerol diluted PA-affinity purified antibody) in PBS-TWEEN containing 0.5% BSA, 0.025% Non-fat milk powder and 100 µM sodium orthovanadate. Incubate with continuous shaking at room temperature for one hour.

10 9. Wash plates 4X with PBS-TWEEN.

10. Add 100µl HRP-linked Ig (1:5,000) in PBS-TWEEN containing 0.5% BSA, 0.025% Non-fat milk powder, 100 µM sodium orthovanadate. Incubate with shaking at room temperature for one hour.

15 11. Wash plates 4X with PBS-TWEEN and then once with PBS.

12. Develop plate using ABTS or other peroxidase substrate.

CELL CYCLE ASSAY:

A431 cells in standard growth medium are exposed to a
20 desired concentration of a test compound for 20-24 hours at 37 °C. The cells are then collected, suspended in PBS, fixed with 70% ice-cold methanol and stained with propidium iodide.

The DNA content is then measured using a FACScan flow
cytometer. Cell cycle phase distribution can then be
25 estimated using CellFIT software (Becton-Dickinson).

HUV-EC-C ASSAY

This assay is used to measure a compound's activity
against PDGF-R, FGF-R, VEGF, aFGF or Flk-1/KDR, all of which
are naturally expressed by HUV-EC cells.

30 DAY 0

1. Wash and trypsinize HUV-EC-C cells (human umbilical vein endothelial cells, (American Type Culture Collection, catalogue no. 1730 CRL). Wash with Dulbecco's phosphate-buffered saline (D-PBS, obtained from Gibco BRL, catalogue no.

14190-029) 2 times at about 1 ml/10 cm² of tissue culture flask. Trypsinize with 0.05% trypsin-EDTA in non-enzymatic cell dissociation solution (Sigma Chemical Company, catalogue no. C-1544). The 0.05% trypsin is made by diluting 0.25%
5 trypsin/1 mM EDTA (Gibco, catalogue no. 25200-049) in the cell dissociation solution. Trypsinize with about 1 ml/25-30 cm² of tissue culture flask for about 5 minutes at 37°C. After cells have detached from the flask, add an equal volume of assay medium and transfer to a 50 ml sterile centrifuge tube (Fisher
10 Scientific, catalogue no. 05-539-6).

2. Wash the cells with about 35 ml assay medium in the 50 ml sterile centrifuge tube by adding the assay medium, centrifuge for 10 minutes at approximately 200x g, aspirate the supernatant, and resuspend with 35 ml D-PBS. Repeat the
15 wash two more times with D-PBS, resuspend the cells in about 1 ml assay medium/15 cm² of tissue culture flask. Assay medium consists of F12K medium (Gibco BRL, catalogue no. 21127-014) and 0.5% heat-inactivated fetal bovine serum. Count the cells with a Coulter Counter® (Coulter Electronics, Inc.) and add
20 assay medium to the cells to obtain a concentration of 0.8-1.0 x 10⁵ cells/ml.

3. Add cells to 96-well flat-bottom plates at 100 µl/well or 0.8-1.0 x 10⁴ cells/well, incubate ~24h at 37°C, 5% CO₂.

25 **DAY 1**

1. Make up two-fold test compound titrations in separate 96-well plates, generally 50 µM on down to 0 µM. Use the same assay medium as mentioned in day 0, step 2 above. Titrations are made by adding 90 µl/well of test compound at
30 200 µM (4X the final well concentration) to the top well of a particular plate column. Since the stock test compound is usually 20 mM in DMSO, the 200 µM drug concentration contains 2% DMSO.

A diluent made up to 2% DMSO in assay medium (F12K + 0.5%

fetal bovine serum) is used as diluent for the test compound titrations in order to dilute the test compound but keep the DMSO concentration constant. Add this diluent to the remaining wells in the column at 60 µl/well. Take 60 µl from the 120 µl of 200 µM test compound dilution in the top well of the column and mix with the 60 µl in the second well of the column. Take 60 µl from this well and mix with the 60 µl in the third well of the column, and so on until two-fold titrations are completed. When the next-to-the-last well is mixed, take 60 µl of the 120 µl in this well and discard it. Leave the last well with 60 µl of DMSO/media diluent as a non-test compound-containing control. Make 9 columns of titrated test compound, enough for triplicate wells each for: (1) VEGF (obtained from Pepro Tech Inc., catalogue no. 100-200, (2) endothelial cell growth factor (ECGF) (also known as acidic fibroblast growth factor, or aFGF) (obtained from Boehringer Mannheim Biochemica, catalogue no. 1439 600), or, (3) human PDGF B/B (1276-956, Boehringer Mannheim, Germany) and assay media control. ECGF comes as a preparation with sodium heparin.

2. Transfer 50 µl/well of the test compound dilutions to the 96-well assay plates containing the $0.8-1.0 \times 10^4$ cells/100 µl/well of the HUV-EC-C cells from day 0 and incubate ~2 h at 37° C, 5% CO₂.

3. In triplicate, add 50 µl/well of 80 µg/ml VEGF, 20 ng/ml ECGF, or media control to each test compound condition.

As with the test compounds, the growth factor concentrations are 4X the desired final concentration. Use the assay media from day 0 step 2 to make the concentrations of growth

factors. Incubate approximately 24 hours at 37° C, 5% CO₂.

Each well will have 50 µl test compound dilution, 50 µl growth factor or media, and 100 µl cells, which calculates to 200 µl/well total. Thus the 4X concentrations of test compound

and growth factors become 1X once everything has been added to the wells.

DAY 2

1. Add ³H-thymidine (Amersham, catalogue no. TRK-686) at 1 µCi/well (10 µl/well of 100 µCi/ml solution made up in RPMI media + 10% heat-inactivated fetal bovine serum) and incubate ~24 h at 37°C, 5% CO₂. RPMI is obtained from Gibco BRL, catalogue no. 11875-051.

DAY 3

1. Freeze plates overnight at -20°C.

DAY 4

- Thaw plates and harvest with a 96-well plate harvester (Tomtec Harvester 96®) onto filter mats (Wallac, catalogue no. 1205-401), read counts on a Wallac Betaplate™ liquid scintillation counter.

IN VIVO ANIMAL MODELS ASSAYS

Xenograft animal models

- The ability of human tumors to grow as xenografts in athymic mice (e.g., Balb/c, nu/nu) provides a useful in vivo model for studying the biological response to therapies for human tumors. Since the first successful xenotransplantation of human tumors into athymic mice, (Rygaard and Povlsen, 1969, Acta Pathol. Microbial. Scand. 77:758-760), many different human tumor cell lines (e.g., mammary, lung, genitourinary, gastro-intestinal, head and neck, glioblastoma, bone, and malignant melanomas) have been transplanted and successfully grown in nude mice. The following assays may be used to determine the level of activity, specificity and effect of the different compounds of the present invention. Three general types of assays are useful for evaluating compounds: cellular/catalytic, cellular/biological and in vivo. The object of the cellular/catalytic assays is to determine the effect of a compound on the ability of a TK to phosphorylate

tyrosines on a known substrate in a cell. The object of the cellular/biological assays is to determine the effect of a compound on the biological response stimulated by a TK in a cell. The object of the in vivo assays is to determine the effect of a compound in an animal model of a particular disorder such as cancer.

Suitable cell lines for subcutaneous xenograft experiments include C6 cells (glioma, ATCC # CCL 107), A375 cells (melanoma, ATCC # CRL 1619), A431 cells (epidermoid carcinoma, ATCC # CRL 1555), Calu 6 cells (lung, ATCC # HTB 56), PC3 cells (prostate, ATCC # CRL 1435), SKOV3TP5 cells and NIH 3T3 fibroblasts genetically engineered to overexpress EGFR, PDGFR, IGF-1R or any other test kinase. The following protocol can be used to perform xenograft experiments:

Female athymic mice (BALB/c, nu/nu) are obtained from Simonsen Laboratories (Gilroy, CA). All animals are maintained under clean-room conditions in Micro-isolator cages with Alpha-dri bedding. They receive sterile rodent chow and water ad libitum.

Cell lines are grown in appropriate medium (for example, MEM, DMEM, Ham's F10, or Ham's F12 plus 5% - 10% fetal bovine serum (FBS) and 2 mM glutamine (GLN)). All cell culture media, glutamine, and fetal bovine serum are purchased from Gibco Life Technologies (Grand Island, NY) unless otherwise specified. All cells are grown in a humid atmosphere of 90-95% air and 5-10% CO₂ at 37°C. All cell lines are routinely subcultured twice a week and are negative for mycoplasma as determined by the Mycotect method (Gibco).

Cells are harvested at or near confluency with 0.05% Trypsin-EDTA and pelleted at 450 x g for 10 min. Pellets are resuspended in sterile PBS or media (without FBS) to a particular concentration and the cells are implanted into the hindflank of the mice (8 - 10 mice per group, 2 - 10 x 10⁶ cells/animal). Tumor growth is measured over 3 to 6 weeks using venier calipers. Tumor volumes are calculated as a

product of length x width x height unless otherwise indicated. P values are calculated using the Students t-test. Test compounds in 50 - 100 μ L excipient (DMSO, or VPD:D5W) can be delivered by IP injection at different concentrations generally starting at day one after implantation.

Tumor invasion model

The following tumor invasion model has been developed and may be used for the evaluation of therapeutic value and efficacy of the compounds identified to selectively inhibit KDR/FLK-1 receptor.

Procedure

8 week old nude mice (female) (Simonsen Inc.) are used as experimental animals. Implantation of tumor cells can be performed in a laminar flow hood. For anesthesia, Xylazine/Ketamine Cocktail (100 mg/kg ketamine and 5 mg/kg Xylazine) are administered intraperitoneally. A midline incision is done to expose the abdominal cavity (approximately 1.5 cm in length) to inject 10^7 tumor cells in a volume of 100 μ l medium. The cells are injected either into the duodenal lobe of the pancreas or under the serosa of the colon. The peritoneum and muscles are closed with a 6-0 silk continuous suture and the skin is closed by using wound clips. Animals are observed daily.

Analysis

After 2-6 weeks, depending on gross observations of the animals, the mice are sacrificed, and the local tumor metastases to various organs (lung, liver, brain, stomach, spleen, heart, muscle) are excised and analyzed (measurement of tumor size, grade of invasion, immunochemistry, in situ hybridization determination, etc.).

ADDITIONAL ASSAYS

Additional assays which may be used to evaluate the compounds of this invention include, without limitation, a

bio-flk-1 assay, an EGF receptor-HER2 chimeric receptor assay in whole cells, a bio-src assay, a bio-lck assay and an assay measuring the phosphorylation function of raf. The protocols for each of these assays may be found in U. S.

5 Application Ser. No. 09/099,842, which is incorporated by reference, including any drawings, herein.

B. Examples - Biological Activity.

10 Examples of the in vitro potency of compounds of this invention are shown in Table 2.

TABLE 2

Example	bio FGFR1 IC50 (mM)	bio flkGST IC50 (mM)	bio EGFR IC50 (mM)	bio PDGFR IC50 (mM)	cdk2SPA IC50 (mM)
1	0.22	0.05		2.54	
2	4.88	0.12	>20	14.59	0.03
3	5.57	1.09	>20	>20	0.08
4	>20	0.009	>20	2.03	0.75
5	1.18	0.035	>20	7.57	
6	1.6	0.009	>20	0.73	
7	1.32	0.028	>20	0.73	
8	7.01	6.07	>20	>20	
9	11.04	1.14	>20	8.77	
10	1.25	0.07	>20	0.39	
11	10.91	1.41	>20	9.29	
12	1.1	0.49	16.33	5.47	
14	0.03	0.06	17.95	14.37	
15	>20	>20	>20	>20	
16	5.2	7.91	>20	1.02	
17	2.08	0.57	>20	1.74	
18	0.08	0.02	>20	0.39	
19	0.87	0.19	>20	8.61	
29	3.83	0.71	>20	1.88	0.01
30	1.34	0.07	>20	1.23	0.02

31	0.98	0.06	>20	1.5	0.02
32	2.09	0.51	>20	1.21	0.009
33					0.06
34					0.26
35	1.65	0.14			
36	3.78	0.03			
37	1.31	0.12			
38	2.18	0.18	>20		
39	16.4	1.24			
40	5.26	0.32	>20	1.7	
41	13.52	5.94	>20	>20	
42	0.31	0.17	>20	1.78	
43	0.24	0.07	>20	0.69	
44	0.05	0.03	19.13	0.4	1.96
45	>20	>20	>20	>20	
46	19.09	5.03	>20	>20	
47	1.1	0.24	>20	3.28	
48	0.26	0.02	>20	0.15	
49	3.7	14.64	>20	4.63	
50	2.81	4.41	14.74	4.89	
51	0.22	0.01	>20	2.31	
52	10.09	10.5	>20	19.05	
53	2.24	1.51	>20	13.38	
54	>20	>20	>20	>20	
55	2.08	1.44	>20	3.84	
60	8.51	1.97	>20	10.38	
61	4.57	0.62	>20	12.54	
62	0.92	0.07	>20	1.47	
63	1.22	0.21	>20	6.58	
64	1.19	0.45	>20	4.57	
65	2.55	0.78	>20	14.36	
66	0.19	0.018	>20	2.1	
67	0.53	0.036	>20	1.68	

68	1.18	0.16	>20	3.37	
69	1.08	0.02	>20	2.04	
70	19.95	3.1	>20	5.6	
71	2.19	0.43	>20	7.51	
72	1.23	0.008	>20	1.77	

7. MEASUREMENT OF CELL TOXICITY

Therapeutic compounds should be more potent in inhibiting receptor tyrosine kinase activity than in exerting a cytotoxic effect. A measure of the effectiveness and cell toxicity of a compound can be obtained by determining the therapeutic index, i.e., IC_{50}/LD_{50} . IC_{50} , the dose required to achieve 50% inhibition, can be measured using standard techniques such as those described herein. LD_{50} , the dosage which results in 50% toxicity, can also be measured by standard techniques as well (Mossman, 1983, J. Immunol. Methods, 65:55-63), by measuring the amount of LDH released (Korzeniewski and Callewaert, 1983, J. Immunol. Methods, 64:313, Decker and Lohmann-Matthes, 1988, J. Immunol. Methods, 115:61), or by measuring the lethal dose in animal models. Compounds with a large therapeutic index are preferred. The therapeutic index should be greater than 2, preferably at least 10, more preferably at least 50.

CONCLUSION

It would be appreciated that the compounds, methods and pharmaceutical compositions of the present invention are effective in modulating PK activity and therefore are expected to be effective as therapeutic agents against RTK, CTK-, and STK-related disorders.

One skilled in the art would also readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent herein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred

embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention are defined
5 by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

10 All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and
15 individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the
20 terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions
25 of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed
30 by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

35 In addition, where features or aspects of the invention

are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group. For example, if X is described
5 as selected from the group consisting of bromine, chlorine, and iodine, claims for X being bromine and claims for X being bromine and chlorine are fully described.

Other embodiments are within the following claims.